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## ERRATA

## Vol. LXXXIV

- P. 354, line 30, for "PACTON" read "PAXTON"
- P. 364, line 18, for "(20)" read "(21)"
- P. 377, line 8 (center heading) for "18." read "19."
- P. 379, line 4, for "ventral" read "dorsal"

## Vol. LXXXV

- P. 27, line 7, for "WIAMEANA" read "WAIMEANA"
- P. 46, line 6 from bottom, for "only very slightly" read "not at all"
- P. 129, line 4, for "acid phosphate" read "superphosphate"
- P. 129, line 9 from bottom, for "tested" read "test"
- P. 143, line 27, for "culm was greater" read "culm wall was greater"





# THE BOTANICAL GAZETTE

March 1928

## STUDIES IN THE GENUS *BIDENS*. VIII

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 375

EARL EDWARD SHERFF

(WITH PLATES I-V)

*BIDENS PILOSA* var. *ALAUSENSIS* (H.B.K.) Sherff (BOT. GAZ. 81:35. 1926) and certain related material.—

In a former article (BOT. GAZ. 76:158. 1923), *Bidens chilensis* var. *apiifolia* DC. was discussed and the Berlin cotype (*Poeppig* 207) of that variety was treated as "an unimportant foliage form of *B. alausensis*," later reduced by me to *B. pilosa* var. *alausensis*. Subsequent study of much cotype material, in conjunction with several specimens collected for me by J. Francis McBride at Matucana, Peru, revealed closer affinities of several of the original specimens with *B. pilosa* var. *radiata* Schz. Bip. Specimens of *Poeppig* 207 were studied in collections belonging to several of the older herbaria (Hb. Univ. Halle; Hb. DC. in Hb. Deless.; Hb. Mus. Vienna; Hb. Univ. Kiel; Hb. Berl., etc.). Two specimens in Vienna and one in Berlin appeared closer to var. *alausensis*, but the cotypes elsewhere appeared, when studied in the light of the interesting McBride specimens previously described by me (cf. BOT. GAZ. 81:35. 1926), to be closer to var. *radiata*.

The nomenclatural problem offered by the publication in 1836, by DE CANDOLLE, of this var. *apiifolia* is particularly vexing, since its publication antedated that for both the var. *alausensis* and the var. *radiata*. An equation of the var. *apiifolia* with either of these two varieties would compel, therefore, the taking up of the name *apiifolia* and the reduction of a well established name to synonymy. The intermediate nature and ill defined status of the variety *apiifolia* would make such a course seem unwise, if not indeed impossible (cf. Internat. Rules Bot. Nomencl. Art. 51, no. 4. 1906). Apparently the interests of taxonomy are best conserved by employing the new combination *B. pilosa* var. *apiifolia* (DC.), to apply only to the form intermediate between

vars. *alausensis* and *radiata*, and represented by Poeppig 207. In this case, the last two names, each certain in its application and well supported in herbaria by illustrative material, are left undisturbed.<sup>1</sup>

**BIDENS ANDICOLA** var. **cosmantha** (Griseb.) comb. nov.; *B. cosmantha* Griseb., Goett. Abhandl. 19:137. 1874.—

GRISEBACH's type of *B. andicola* var. *cosmantha* was *P. G. Lorentz* 1526, La Cienaga, Sierra de Tucuman, Argentina, March 25-31, 1872 (Hb. Berl.). Identical material was collected by *Lorentz* under nos. 540 and 540b (Granadillas near Yacatula, Province of Catamarca, Argentina, beginning of February, 1872; Hb. Berl.). These Lorentzian specimens are connected with *B. andicola* H.B.K. by too many intermediate forms to permit their being regarded as specifically distinct. They may best be regarded as a variety of *B. andicola*, more or less distinguished from the species proper by the tall slender habit, by the leaves more dissected (but less so than in var. *decomposita* O. Ktze.), and with outlines suggesting those of celery (*Apium graveolens* L.), also by the larger flowering heads, which when fully expanded reach a diameter of 6-7 cm.

**BIDENS ANDICOLA** H.B.K. Nov. Gen. et Sp. 4:186 (237). 1820; *B. cosmantha* var. *diversifolia* Griseb. Symbol. Argent. 198. 1879.—

GRISEBACH's *B. cosmantha* var. *diversifolia* was described as having the lowermost leaves ternatisect, the others entire, elliptic-oblong, sharply serrate. It came from the Province of Catamarca, Argentina. The Catamarca plants studied by GRISEBACH had been collected by *F. Schickendantz* (Griseb. loc. cit. 3). A good specimen of *F. Schickendantz* 200, from Catamarca and bearing the determination *B. cosmantha* var. *diversifolia* Griseb., is extant in the Berlin Herbarium. It is merely a specimen of *B. andicola* H.B.K.

**BIDENS ASYMMETRICA** (Lévl.) Sherff, BOT. GAZ. 81:49. 1926; *B. gracilis* Nutt., Trans. Amer. Phil. Soc. Ser. II. 7:368. 1841; Sherff, loc. cit. 70:105 and Pl. XIII. 1920; non *B. gracilis* Torr., Ann. Lyc. N.Y. 2:215. 1828; *Campylothea gracilis* (Nutt.) Walp. Repert. Bot. Syst. 2:618. 1843; *Lipochaeta asymetrica* Lévl., Fedde Repert. Sp. Nov. 10:122. 1912 (Pl. III).—

Excellent specimens of the type number (*Abbé Urbain Faurie* 960) of *B. asymetrica* are preserved in herbaria (Hb. Par.; Hb. Deless.; Hb. Brit. Mus.). From a study of these specimens, it is evident that *B. asymetrica* is specifically the same as *B. gracilis* Nutt., being merely a coarser and more herbaceous form of that species.

<sup>1</sup> Concerning the intermediate nature of var. *apiifolia*, it may be remarked that while DE CANDOLLE ranked this variety under *B. chilensis* DC. (= *B. pilosa* var. *alausensis*), Poeppig's original printed labels bore the name *B. leucantha* L. (= *B. pilosa* var. *radiata*).

Recently Professor OTTO DEGENER, of the University of Hawaii, has very kindly written me that *Bidens gracilis* Nutt. should be dropped, in view of the earlier [but invalid] name *B. gracilis* Torr. DEGENER proposed a new name. Since, moreover, it is likely that the International Rules of Nomenclature will ere long be amended in such manner as to compel (as does the American Code now) the retirement of such names as *B. gracilis* Nutt., DEGENER's proposal would seem at first a good one. In our present case, however, the synonym *B. asymmetrica* is next in line for adoption, and precludes the necessity of using a new binomial as proposed by him.

***Bidens degeneri*** nom. nov.; *Campylotheca dichotoma* Hillebr. Fl. Hawaiian Isls. 212. 1888; *Bidens dichotoma* (Hillebr.) Sherff, Bot. GAZ. 70:98. 1920; non *B. dichotoma* Desf. Tabl. Edit. I:108. 1804 (*nom. nud.*); et Cat. Pl. Hort. Reg. Par. Edit. III:185. 1829.—

This species is here renamed to avoid the confusion consequent upon the existence of the earlier *Bidens dichotoma* Desf. In choosing the trivial name it is a pleasure to acknowledge the assistance received from Professor DEGENER, who has placed at my disposal several independent observations on the nomenclature of certain Hawaiian species, and has obtained valuable herbarium specimens for my studies.

**BIDENS DEGENERI apioides** var. nov.—E specie foliis plerumque 3—partitis, grosse serratis vel dentatis vel etiam lobatis, foliolo terminali interdum subtripartito differt.

*Otto Degener* and *Henry Wiebke* 2179—b, wind-swept ridge, north of Pohakea Gulch, near Wailuku, Maui, Hawaiian Isls., July 23, 1927 (Hb. Field Mus., type; Hb. Univ. Hawaii).

The type of *B. degeneri* was known to have been collected upon Maui (*cf.* Hillebrand Fl. Hawaiian Isles. 213. 1888) but whether from "above Maalaea or on Haleakala" was not known definitely. HILLEBRAND (*loc. cit.*) cited *Mann* and *Brigham* 450 from a mountain near Waikapu, of the same island. *Gaudichaud* had collected this species somewhere in the Hawaiian Islands during September and October, 1836 (Hb. Par.). It has since been collected upon Oahu (*C. N. Forbes* 1982 O, Waimano Ridge, October 27-30, 1914, Hb. Bishop Mus.), upon Molokai (*C. N. Forbes*, sea cliffs of Halawa Valley, August, 1912, Hb. Bishop Mus.), and again upon Maui (*Degener* and *Wiebke* 2179, arid, wind-swept ridge, north of Pohakea Gulch, near Wailuku, July 23, 1927, Hb. Field Mus.; Hb. Berl.; Hb. Boiss.; Hb. Univ. Hawaii; Hb. Kew).

Western Maui is connected with eastern Maui by a broad, sandy isthmus, Wailuku, which appears to be the real home of the species. Recently *Degener* and *Wiebke*, on finding many specimens of the species proper, their no. 2179, growing near Wailuku, observed several bizarre plants which may be designated 2179-b. Regarding these latter, Professor DEGENER (*in litt.*) states: "Plants

of this type are somewhat rare and grow intermingled with typical ones." The leaves have their leaflets deeply toothed or lobed and suggest in outline the leaves of common celery, *Apium graveolens* L., whence the varietal name selected by me, *apioides*.

Among the specimens collected by *Degener* and *Wiebke* is a series of plants from the Island of Maui, their number 2163. These, while appearing new, are too close to *B. campylothea* Schz. Bip. to warrant being ranked as specifically distinct. Their principal leaves, however, are regularly 5-parted, while in the species proper the leaves of well developed plants are regularly 3-parted.<sup>2</sup> The flowering heads have about 8 long, linear ligules, and when fully expanded reach a diameter of 4 cm. In the species proper, the ligules are usually fewer, shorter and broader, and the flowering heads commonly measure about 2 cm. in diameter. Heretofore the species has been definitely known only from the Islands of Oahu, Lanai, and Hawaii. The new form from Maui appears best treated as a variety:

**BIDENS CAMPYLOTHECA pentamera** var. nov.—Folia principalia plerumque 5—partita, jugi inferioris foliolis lanceolatis, sessilibus vel vix petiolulatis, circ. 5–6.6 cm. longis et 1.4–2.9 cm. latis; capitulis pansis ad anthesin 3–4 cm. latis; floribus ligulatis circ. 8, ligulis linearibus. Achaenia torta, non nisi ad apicem setosa, exaristata vel rariter obsolete aristata, 1 vel 2 aristis minutis, glabris, etiam infra apicem positis et in achaeniorum margines decurrentibus.

*Otto Degener* and *Henry Wiebke* 2163, fog-swept medium forest in Koolau Gap, Haleakala Crater, Maui, Hawaiian Isls., August 11, 1927 (type and cotypes in Hb. Field Mus.; cotypes in Hb. Univ. Hawaii; Hb. Berl.; Hb. Brit. Mus.; Hb. Par.; Hb. Kew, etc.).

**BIDENS CAMPYLOTHECA** var. **PENTAMERA** f. **filicifolia** f. nov.—E varietate foliis 7–9—foliolatis, foliolis terminalibus et basalibus saepe irregulariter 2–5—partitis, aliis simplicibus differt.

<sup>2</sup> I have been fortunate in securing a good representation of *B. campylothea* in herbarium specimens for study: *Otto Degener* and *Henry Wiebke* 3000, sunny, rocky slope, Punaluu Valley, Isl. Oahu, Feb. 20, 1927 (Hb. Field Mus.; Hb. Univ. Haw.), *C. N. Forbes* 195H, Hanehane, Kona, Isl. Hawaii, June 17, 1911 (Hb. Bishop Mus.), *idem* 346H, Honomalino, Kona, Isl. Hawaii, July 25, 1911 (Hb. Bishop Mus.); *Gaudichaud* (Voyage of the Bonite) 220 *pro parte* and 221 *pro parte*, Hawaiian Isls., September–October, 1836 (Hb. Par.); *idem*, Hawaiian Isls., October, 1836 (Hb. Deless.); *Dr. W. Hillebrand* 39, Isl. Hawaii (Hb. Brit. Mus.; Hb. Kew); *Macrae*, Isl. Oahu, May, 1825 (Hb. Kew); *idem*, at Mt. Kaah, Isl. Oahu, June, 1825 (Hb. Deless.; Hb. Kew); *G. C. Munro* 464, Kaiholena, Isl. Lanai, Aug. 16, 1915 (Hb. Bishop Mus.); *idem* 505; Kohinahuia, Isl. Lanai, Feb. 12, 1916 (Hb. Bishop Mus., 3 sheets); *J. Remy* 285, Isl. Hawaii, 1851–1855 (Hb. Paris).

<i>Prunus demissa</i> (Nutt.) Walp.....	Chokecherry
<i>Pteridium aquilinum</i> (L.) Kuhn.....	Brake fern
<i>Rosa nutkana</i> Pres.....	Wild rose
<i>Salix scouleriana</i> Barr.....	Willow
<i>Scritiotheca dumosa</i> (Nutt.) Rydb. (= <i>Holodiscus dumosus</i> (Nutt.) Heller).....	Ocean spray
<i>Sedum douglasii</i> Hook.....	Stone crop
<i>Senecio</i> sp.....	Groundsel
<i>Spiraea lucida</i> Doug.....	Spiraea
<i>Zygadenus venenosus</i> S. Wats.....	Death camas

### WESTERN LARCH-DOUGLAS FIR FORESTS

The larch and Douglas fir forests grow under somewhat better moisture conditions and a little lower temperature than the forests of yellow pine, and the trees stand closer together, so that the associated vegetation is found in the natural openings.

#### *Associated Species*

<i>Amelanchier alnifolia</i> Nutt. (= <i>A. florida</i> Lindl.).....	June berry
<i>Antennaria racemosa</i> Hook.....	Everlasting
<i>Antennaria rosea</i> (D. C. Eat.) Greene.....	Everlasting
<i>Arctostaphylos uva-ursi</i> (L.) Spreng.....	Kinnikinnick
<i>Castilleja miniata</i> Dougl.....	Indian paint-brush
<i>Ceanothus velutinus</i> Dougl.....	Mountain balm
<i>Chimaphila umbellata</i> (L.) Nutt.....	Princess pine
<i>Cythrea bulbosa</i> (L.) House (= <i>Calypso borealis</i> Salish.).....	Calypso
<i>Drymocalis glandulosa</i> (Lindl.) Rydb.....	Drymocalis
<i>Fragaria</i> sp.....	Wild strawberry
<i>Lepargyrea argentea</i> (Nutt.) Greene.....	Silver brush
<i>Lepargyrea canadensis</i> (L.) Greene.....	Buffalo berry
<i>Linnaea borealis</i> L.....	Twin flower
<i>Lonicera utahensis</i> S. Wats.....	Honeysuckle
<i>Odostemon repens</i> (Lindl.) Cockerell (= <i>Berberis repens</i> Lindl.).....	Oregon grape
<i>Pachystima myrsinites</i> Raf.....	Goat brush
<i>Smyphoricarpus racemosus</i> Michx.....	Snowberry
<i>Sorbus angustifolia</i> Rydb.....	Mountain ash.

### WHITE PINE, CEDAR, HEMLOCK, AND LOWLAND WHITE FIR FORESTS

The trees which make up the white pine, cedar, hemlock, and lowland white fir belt are found on deeper soils with greater moisture-holding capacity than either of the two aforementioned, and their characteristic sites are steeper north slopes, fertile lower slopes, benches, and bottoms within the more mountainous regions. Here the extremes of the climate are reduced by the greater precipitation, cloudiness, the denser stand and consequent shade. There is, therefore, much more material for decay, and an accumulation of humus and litter where the moisture-holding capacity of the soil is improved.

In this forest belt not only a greater variety



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*Otto Degener* and *Henry Wiebke* 2177 *pro parte*, Koolau Gap, Haleakala Crater, Maui, Hawaiian Isls., August 11, 1927 (type and cotypes in Hb. Field Mus., cotypes in Hb. Univ. Hawaii).

One specimen of *Degener* and *Wiebke* 2177 is typical for the var. *pentamera*, but the remaining ones differ conspicuously in having 7-9 leaflets, the terminal one and the two of the basal pair often irregularly 2-5 parted. In view of the pronounced endemism among the Hawaiian plants, it seems wise to interpret this distinction as connoting a definite *forma* of *B. campylotheca* var. *pentamera*.

***Bidens ctenophylla* sp. nov.**—Frutex ramosus ramis rubescentibus subtetragonalibus et vix glaucescentibus, glabratis, verisimiliter 5-10 dm. altus. Folia petiolata petiolis tenuibus 2-5 cm. longis, petiolo adjecto 5-12 cm. longa, membranacea, plerumque simplicia, basim integram versus rotundata vel late cuneata, supra medium raro integra plerumque perspicue pectinato-dentata dentibus (in unico latere saepius 3-9) plerumque prorsum spectantibus, apice acuminata, circumambitu ovata vel rhomboideo-ovata, margine aegre ciliata, supra glabrata, infra saepe paucis setis sparsissime adpresso-hispida; rarius tripartita, foliolis lanceolatis, lateralibus minoribus. Capitula numerosa, in inflorescentia corymbiformi disposita, radiata, pañsa ad anthesin 4-6 mm. alta et circ. 1.5-1.8 cm. lata; pedicellis et pedunculis minimis dense et insigniter albido-pubescentibus. Involucri bractee dense hispidae, exteriores 5-8, lineares, patentes, apice acutae, 1.5-2.5 mm. longae; interiores lanceolatae, dimidio longiores. Flores ligulati plerumque 5, ligula late lineari-oblongi vel obovati, flavi, apice rotundato integri vel obscure denticulati, 7-11 mm. longi. Achaenia demum paleas anguste lineares paulo superantia, recta vel subrecta, sublata oblongo-lineararia, valde obcompressa, corpore nigro 8-11 mm. longa et 1.5-2 mm. lata, faciebus marginibusque glabra, apice (saepe imperfecte) biaristata aristis usque ad 1.2 mm. longis in achaeniorum duos subalatos stramineos margines decurrentibus, retrorsum barbatis vel demum plus minusve calvis.

*Otto Degener* and *Henry Wiebke* 2128, arid, weed-covered "aa"<sup>3</sup> slopes, between Puuwaawaa and Huehue, Hawaii, Hawaiian Islands, August 22, 1926 (type and cotypes in Hb. Field Mus.; cotypes in Hb. Univ. Hawaii, Hb. Berl., Hb. Brit. Mus., Hb. Par., Hb. Kew., etc).

In foliage this species resembles more strongly the simple-leaved forms of *B. asymmetrica* (Lévl.) Sherff (*B. gracilis* Nutt.) than it does any other Hawaiian

<sup>3</sup> *Aa*, anglicized from the Hawaiian and meaning a loose, rough type of lava (*vide* O. Degener *in litt.*).

species of *Bidens*. The teeth are frequently so elongated as to give a comblike or pectinate edge to the leaves. The branching habit also is like that of *B. asymmetrica*. The capitula, however, are more numerous than in *B. asymmetrica*, and are borne on conspicuously white-pubescent branchlets. The achenes, furthermore, are distinct, being wider and noticeably broad-margined, the winglike margins continuing at the top into the aristae. In fact, the achenes suggest somewhat those of *B. macrocarpa* (Gray) Sherff.

***Bidens hillebrandiana*** (Drake del Cast.) Deg. *in litt.*, comb. nov.; *Campylothea remyi* Hillebr. Fl. Hawaii. Isls. 212. 1888; *Coreopsis hillebrandiana* Drake del Cast. Illustr. Fl. Ins. Mar. Pacif. 209. 1890; *Campylothea rutifolia* Lévl., Fedde Repert. Spec. Nov. 10:123. 1911; *Bidens remyi* (Hillebr.) Sherff, Bot. Gaz. 70:97. 1920; *non B. remyi* Drake del Cast. *loc. cit.* 78, pl. 39. 1888; *ibid.* 210. 1890.—

*Bidens remyi* Dr. del Cast. was published at first (1888) only with a plate and analysis. The description followed later (1890). Even though this name (based upon *J. Remy* 281) is referable to *B. micrantha* Gaud. (*cf.* Bot. Gaz. 70:97, footnote 9, 1920), it nevertheless may well be taken as having precluded the acceptance of my name *B. remyi* (Hillebr.), made later. DEGENER suggests (*in litt.*, Feb. 21, 1927) the taking up, therefore, of the trivial name *hillebrandiana* for the plant first described by HILLEBRAND under the name *Campylothea remyi*.<sup>4</sup>

**BIDENS SANDVICENSIS** var. ***setosa*** comb. nov.; *B. setosa* Sherff, Bot. Gaz. 70:103. 1920.—

The type material of *B. setosa* (*C. N. Forbes* 811K, Waimea Drainage Basin, West Side, Kauai, Hawaiian Isls., July 3–August 18, 1917) was a slightly monstrous or pathological form, as to vegetative parts, and did not match any known species. The singularly hairy achenes were very unique and were relied upon as indicating a distinct species. More recently, however, I found in the Delessert Herbarium a specimen from another collection (*Abbé Faurie* 961, Holokele, Hawaii, Hawaiian Isls., March, 1910). This lacked all traces of any pathological condition. Its floral and achenial characters matched those of the type precisely, but the foliage and growth habit were those of normal *B. sandvicensis* Less.<sup>5</sup> I have concluded that a varietal rank under *B. sandvicensis* is more nearly expressive of the true status of the *setosa* forms.

<sup>4</sup> I am informed very kindly by Dr. JOHN HENDLEY BARNHART, of the New York Botanical Garden (*in litt.*, Nov. 30, 1927), that HILLEBRAND'S *Flora Hawaiiensis* was mentioned in the Journal of Botany for April, 1888, and was published "probably in March" of that year. Dr. BARNHART finds DRAKE DEL CASTILLO'S work (Ill. Fl. Ins. Mar. Pacif., fasc. 4) referred to later, Nat. Novit., 2nd number for June, 1888; he believes that DRAKE DEL CASTILLO'S work (fasc. 4) was published "probably late in May" of the same year.

<sup>5</sup> The achenes were mostly exaristate or shortly aristate. A duplicate in the British Museum of Natural History, however, had the achenes mostly very distinctly biaristate with aristae retrorsely barbed and about 1 mm. long.

*BIDENS SANDVICENSIS* var. *caduca* nom. nov.; *Campylotheca sandwicensis* var.  $\beta$ , Hillebr. Fl. Hawaiian Isls. 214. 1888.—

HILLEBRAND (*loc. cit.*) described under *Campylotheca sandwicensis* (Less.) Hillebr. a var.  $\beta$  from the Island of Molokai. This variety, which is here given a more conventional name, is perhaps unrepresented in herbaria. It is of uncertain status. I do not recall having seen authentic material of it and must defer detailed treatment until some future time.

HILLEBRAND's description, here Latinized, is essentially as follows: Folia superiora simplicia vel tripartita, laminis vel segmentis magis elongatis, dentibus minus protractis. Capitula minora et multo plura, inflorescentia corymbiformi sed vix exserta. Achaenia valde exalata, aristis filiformibus vix hamosis et mox caducis.

*BIDENS MACROCARPA* var. *ovatifolia* (Gray) comb. nov.; *B. sandwicensis* var. *ovatifolia* Gray, Proc. Amer. Acad. 5: 128. 1861; *Campylotheca* (*Coreopsis*) *macrocarpa* var. *ovatifolia* (Gray) Hillebr. *loc. cit.* 215.—

GRAY's type (U.S. Nat. Hb.) was collected on the Island of Oahu, by the *United States Exploring Expedition under Captain Wilkes*. I have never seen another specimen exactly matching this type, but *C. N. Forbes* 2313 O, Kalihi, Isl. of Oahu, March 9, 1916 (Hb. Bishop Mus.) shows in its lowermost leaves an approach to the *ovatifolia* form. Both plants have capitula referable to *B. macrocarpa* rather than *B. sandwicensis*. In fact, Drake del Castillo (Illustr. Fl. Ins. Mar. Pacif. 209. 1890) equated the *ovatifolia* form outright with *B. macrocarpa* (*Coreopsis macrocarpa* Gray). The exceptional nature of the leaves, however, makes it seem best to retain a varietal status for Gray's type, as was done by HILLEBRAND.

*BIDENS CAMPYLOTHECA* var. *nematocera* nom. nov.; *Campylotheca grandiflora* var.  $\beta$  Hillebr. *loc. cit.* 215.—

HILLEBRAND retained the name *Campylotheca grandiflora* DC. for the species named *Bidens campylothea* by Schultz Bipontinus (and also, though inadvertently, *Coreopsis macraei* by Gray). Under it he listed one variety, var.  $\beta$ , from the "N. coast of Molokai and probably also of Maui." I have never found authentic material of this variety in herbaria. The description given by HILLEBRAND is essentially as Latinized herewith: Folia petiolata petiolis circ. 7.5 cm. longis, summa indivisa, cetera 3-partita (vel interdum 5-partita, foliolis jugi inferioris breviter petiolulatis, ovato-oblongis et ad basim cuneatis, vel rhomboideis, acute serratis, breviter cuspidatis, 7.6–8.9 cm. longis et 3.2–3.8 cm. latis). Panicula quam ea speciei brevior, cymis axillaribus magis ramosis et rursus paniculatis. Flores ligulati 5–8, ligula obovato-oblongi, 1.5–1.7 cm. longi. Flores disci 15–20. Achaenia angusta, torta, ciliata, biaristata aristis longis (4 mm.), filiformibus, deciduis, erecte retrorsumque dentata.

From the var. *pentamera* already described (*supra* p. 4), the var. *nematocera*

is seen to differ in having the petioles of the principal leaves 7.5 cm. long, not about 2-6 cm. long, the leaflets of the lower pair ovate-oblong with cuneate base, or rhomboideal, shortly petiolulate, 7.6-8.9 cm. long and 3.2-3.8 cm. wide, not lanceolate, not sessile or virtually so, not about 5-6.6 cm. long and 1.4-2.9 cm. wide; the ray florets obovate-oblong, not linear; the achenes ciliate and biaristate with filiform, deciduous awns 4 mm. long, these both upwardly and downwardly toothed [or barbed], not glabrous, nor glabrous except for a few minute bristles at the top, not with the bodies exaristate or sometimes having 1 or 2 minute, smooth awns arising from below the achenial apex and continuous with or decurrent into the achenial margins.

**BIDENS MICRANTHA** var. **laciniata** (Hillebr.) comb. nov.; *Campylothecha micrantha* var. *laciniata* Hillebr. loc. cit. 216.—

In his Flora of The Hawaiian Islands, HILLEBRAND listed two varieties under *Bidens micrantha* Gaud. (*Campylothecha micrantha* Cass). One of these was his var. *laciniata*, and for its habitat the Islands of Maui (Honuaula, Kula, Wailuku) and Hawaii were cited. Fortunately there still exists in the Herbarium of the Bernice Pauahi Bishop Museum (Honolulu) a specimen collected by Hillebrand and Lydgate at Kula, Isl. of Maui, and determined on the original label as “ $\beta$  var. *laciniata* Hbd.” Still another original specimen is in the British Museum of Natural History. It was collected by Hillebrand alone, at Wailuku, and was labeled “*Campylothecha micrantha*  $\beta$  var.” These plants are seen to come from HILLEBRAND’s cited localities and may be regarded as authentic for the var. *laciniata*. Aided by these specimens and HILLEBRAND’s description, I have assembled the following short list of representative specimens: Abbé Faurie 997, Wailuku, Maui, August, 1909 (Hb. Brit. Mus.); C. N. Forbes and C. M. Cook, Jr., 24 M, Maunahooma, W. Maui, May, 1910 (Hb. Bishop Mus.; Hb. Field Mus.); Dr. William Hillebrand, south ridge of Valley of Wailuku, Maui, August, 1870 (Hb. Brit. Mus.); *idem* and Rev. J. M. Lydgate, Kula, Maui (Hb. Bishop Mus.); J. Remy 280, Maui, 1851-1855 (Hb. Gray).

**BIDENS MICRANTHA** var. **kaalana** nom. nov.; *Campylothecha micrantha* var.  $\gamma$  Hillebr. loc. cit.—

HILLEBRAND cited “Oahu! Kaala, and Waianae range” for his var.  $\gamma$ . In the apparent absence from herbaria of authentic original specimens, it had seemed unwise to attempt taking up this variety and giving it a conventional name. Several years ago, however, Professor C. N. FORBES sent me a large collection of Hawaiian specimens of *Bidens* which he had collected (cf. BOT. GAZ. 70:98. 1920), and among these was one plant from the type locality cited by HILLEBRAND: Makaha Valley, Kaala Range, Oahu, February 12-19, 1909 (now in Hb. Field Mus.). This plant matches HILLEBRAND’s description very closely, and is so different from typical *B. micrantha* that it may well be interpreted as representing a valid variety.

**BIDENS MENZIESII** var. **leptodonta** nom. nov.; *Campylotheca* (*Coreopsis*) *menziesii* var.  $\beta$  Hillebr. loc. cit. 216.—

HILLEBRAND cited only the Island of Molokai for *B. menziesii* proper, but fairly typical material has been collected in the northern part of the Island of Maui (C. N. Forbes, August, 1910, Hb. Bishop Mus.; Hb. Field Mus.). HILLEBRAND recognized two varieties. His var.  $\beta$ , here named *leptodonta*, was cited for eastern Maui and also the Island of Hawaii. His description is faulty, giving the leaves as 2 inches long, the primary segments cut to the rhachis. The leaves on some of his own material are over 5 inches long; only comparatively few of the primary segments are cut to the rhachis, but usually enough are thus deeply cut to impart a distinctive foliage aspect. The following specimens of var. *leptodonta* have been examined:

*C. N. Forbes* 162 H, slopes of Hualalai, Isl. of Hawaii, June 15, 1911 (Hb. Bishop Mus.); *Dr. William Hillebrand* 29, Kohala, Isl. of Hawaii (Hb. Kew); *idem* 298, Isl. of Hawaii (Hb. Kew); *idem*, Isl. of Maui (*sub nom. Campylotheca menziesii*  $\beta$  var., Hb. Brit. Mus.); *Joseph F. Rock* 8310, alt. 6000 ft., Kemole, Mauna Kea, Isl. of Hawaii (Hb. Gray).

**BIDENS MENZIESII** var. **filiformis** nom. nov.; *Campylotheca menziesii* var.  $\gamma$  Hillebr. loc. cit. 216.—

This is the variety having the leaf-divisions very narrow and more or less filiform. The following specimens are typical:

*C. N. Forbes* 877 H, Koehe, Isl. of Hawaii, June 17, 1915 (Hb. Bishop Mus.; Hb. Field Mus.); *Dr. William Hillebrand* 30, Central Plateau, Isl. of Hawaii (Hb. Kew); *idem* and *Rev. J. M. Lydgate*, *cum specie commixt.*, Hawaiian Isls. (Hb. Bishop Mus.); *H. Mann* and *W. T. Brigham*, Central Plateau, Isl. of Hawaii (Hb. Bishop Mus.); *idem* 520, alt. 6000 ft., Hualalai, Isl. of Hawaii (Hb. Bishop Mus.; Hb. Deless.; Hb. Kew; Hb. Missouri Bot. Gard.); *idem* 589, Hawaiian Isls. (Hb. Bishop Mus.); *Joseph F. Rock* 8351, Nohonaohae crater, Isl. of Hawaii, June, 1910 (Hb. Gray).

**BIDENS PAUPERCU** Sherff, BOT. GAZ. 76:158. 1923; *B. ciliata* De Wild., Fedde Repert. Sp. Nov. 13:203. 1914; *non B. ciliata* Hoffmgg. ex Fisch. et Mey. Ind. Sem. Hort. Petrop. 6:46. 1839.—

This little known species was first collected by *J. Bequaert*, no. 302, Elisabethville, Katanga, Belgian Congo, April 4, 1912 (Hb. Bruss., 2 sheets). *Bequaert's* plants were the type material of *B. ciliata* De Wild. In the same year *Ad. Stolz* collected a number of excellent specimens of this species (no. 1442, forest at 900 m. alt., Kyimbila, Nyassaland, July 22, 1912; Hb. Berl.—2 sheets; Hb. Univ. Copenh.; Hb. Deless.; Hb. Mus. Vienna; Hb. Munich). In 1914, *Rev. F. A. Rogers* collected in the type locality for *B. ciliata* De Wild. more robust material (no. 10956, Elisabethville, Belgian Congo, May), a specimen

of which I was permitted in 1924, through the courtesy of Dr. J. BURTT DAVY, to examine and photograph.

These plants are not to be confused with *B. ciliata* Hoffm., which is represented by good material still extant (Hb. Mus. Vienna) and is found to be normal *B. pilosa* L.

*BIDENS PILOSA* L. Sp. Pl. 832. 1753; *B. adhaerescens* Vell. Fl. Flum. 348, 8:t. 88. 1827 (*pro parte*).

A careful study of VELLOZO's description and illustration of his *B. adhaerescens* shows that plant to be none other than *B. pilosa* L. His description of the capitulum, however, is not above criticism. In the short, abridged description at the beginning, we read: "floribus terminalibus, discoideis." This would limit the description, of course, to *B. pilosa* proper. In the detailed description which follows, however, we read: "Corollula composita, quandoque radiata, radius octo ligulatis, albis." Thus it is seen that he really included also the plants with white rays, forms which today are separately treated as *B. pilosa* var. *radiata* Schz. Bip.<sup>6</sup>

*BIDENS MICRANTHOIDES* Sherff, BOT. GAZ. 70:100. 1920; *B. angustifolia* Nutt., Trans. Amer. Phil. Soc. Ser. II. 7:369. 1841; *Campylothecca angustifolia* (Nutt.) Walp. Repert. 2:618. 1843; *non Bidens angustifolia* Lam. Encycl. 1:416. 1783.—

The type of *B. angustifolia* Nutt., collected by Thomas Nuttall himself on the Island of Oahu, Hawaiian Islands, is still preserved (Hb. Brit. Mus.). It is found to be identical with the type and other cited specimens of *B. micranthoides* Sherff, but the name *angustifolia* is here rejected because of the existence of the earlier homonym *B. angustifolia* Lam.<sup>7</sup>

*BIDENS CHINENSIS* (L.) Willd. Sp. Pl. 3. 1719 (*quoad synonymia et herb. Willd. n. 15023, fol. 4*). 1804; *B. robertianifolia* Lévl. et Vant. ex Lévl., Fedde Repert. Nov. Sp. 8:140. 1910.—

LÉVEILLÉ cited as the type for *B. robertianifolia* Lévl. and Vant., a plant collected in Corea by Taquet: Island of Quelpaert, in uncultivated places at Hongno, Sept. 23, 1908. There exists a fine specimen with these data in Geneva (Taquet 969, Hb. Deless.). It is *B. chinensis* (L.) Willd.

*Bidens bipontina* nom. nov.; *B. serrulata* Schz. Bip., Flora 39: 361. 1856; *non* Desf. Cat. Hort. Par. Edit. II:130. 1815; *Coreopsis serrulata* (Schz. Bip.) Benth. and Hook. ex Drake del Cast. Illustr.

<sup>6</sup> For an extended discussion of VELLOZO and his work, see HOOK. Jour. Bot. 4:4. 1842.

<sup>7</sup> A name referred by LAMARK himself (*loc. cit.*) and by A. H. MOORE (Proc. Amer. Acad. 42:528. 1907) to *Spilanthes urens* Jacq.

Fl. Ins. Mar. Pacif. 210. 1890 (*cf.* Fl. Polyn. Fr. 109. 1893); *non* Poir. Encycl. Suppl. 2:352. 1811.—

This species, inadequately described by SCHULTZ BIPONTINUS from a fragmentary specimen, *Ed. Jardin* 132, from the Island of Nukahiva (Marquis Isls.), may here be renamed to avoid confusion with *B. serrulata* Desf.

BIDENS SERRULATA (Poir.) Desf. Tabl. Ecol. Bot. Edit. II:130. 1815; Cat. Hort. Par. Edit. III:186. 1829; *Coreopsis serrulata* Poir. Encycl. Suppl. 2:352. 1811; *Bidens grandiflora* Balb. Cat. Hort. Taur. 19. 1812; *B. grandiflora* Pers. *ex* Balb. *loc. cit.* 18. 1813; *Coreopsis diversifolia* Jacq. Eclog. Pl. 1:80, tab. 54. 1811–1816; *Bidens quinquerradiata* Zea *sede* Jacq. *loc. cit.*; *Cosmea lutea* Sims, Curtis's Bot. Mag. 41: pl. 1689. 1815; *Kerneria serrulata* (Poir.) Cass., Dict. Sci. Nat. 51:474. 1827; *Bidens diversifolia* Hort. *ex* DC. Prodr. 5:602. 1836; *non* Willd. *ex* DC. *loc. cit.*; *B. serrulata* Desf. *ex* DC. *loc. cit.*; *B. grandiflora* var. *diversifolia* (Jacq.) O. Ktze. Rev. Gen. 3<sup>11</sup>:136. 1898; *B. grandiflora* var. *serrulata* (Poir.) O. Ktze. *loc. cit. ex synon. sed excl. plantam Boliviae*.<sup>8</sup>—

In my herbarium determinations of this species thus far I have employed the widely accepted name *B. grandiflora* Balb., a name going back to 1812. The trivial name *serrulata* was employed by POIRET one year earlier, however, and must be taken up. Certain authentic specimens of POIRET's species are still extant at Paris, where at least for several years this plant was cultivated. One of them ("Du Jardin des Plantes de Paris. 5 Aout 1813") was photographed by me. They are specifically the same as the plants cultivated by the younger JACQUIN in 1810 from seed sent from Paris (Mus. His. Nat., located in the Jardin des Plantes), and named by him, *Coreopsis diversifolia*. They are matched specifically also by the type illustration of *Cosmea lutea* Sims.

The cultivated specimens found in herbaria are mostly lower, more delicate, and less erect, giving an illusory appearance of dissimilarity to the spontaneous form. POIRET's description was based upon one of DESFONTAINES' specimens. Like many of the descriptions written in those days, it was inadequately drawn. The fruiting characters were omitted. We read that the plant described is small, with petioles and lower part of stem pilose, also with ovate leaves. These last were not described as compound.

DE CANDOLLE (*loc. cit.*), evidently relying solely upon POIRET's description,<sup>9</sup> maintained *B. grandiflora* apart from *B. serrulata* (Poir.) Desf. The Index

<sup>8</sup> KUNTZE's specimen (*ipse leg.*) came from 2100 m. alt., Santa Rosa, Bolivia, April 1, 1892. It is still extant (Hb. N.Y. Bot. Gard.) and is true *Bidens andicola* H.B.K.

<sup>9</sup> "Ab hac (*B. grandiflora* Balb.) *ex* descr. videtur diversa *Coreopsis serrulata* Poir. suppl. 3 [*sic*] p. 352 etiamsi a cl. Desf. admissa."



Kewensis (1:617. 1895), however, equates *Coreopsis serrulata* Poir. and *Bidens grandiflora* Balb. Furthermore, we are bound to consider the very careful and extended description of the Poiret species drawn up by the eminent CASSINI (*loc. cit.*) when publishing the new combination *Kerneria serrulata*. CASSINI had been in Paris, at the center of DESFONTAINES' and also of POIRET's activities. He amplified his description from living material growing in the Royal Garden of Paris, where DESFONTAINES had worked. His description fitted very well the average plants of *B. grandiflora*.

Upon the grounds of priority the name *B. serrulata* goes back to some time in the year 1811 prior to Nov. 29,<sup>10</sup> while *B. grandiflora* was not published until in 1812. The exact date for *Coreopsis diversifolia* Jacq. I do not know. The page number and plate number would seem clearly to indicate, however, a date later than 1811. At all events, the existence of a *B. diversifolia* Willd. (*ex. DC. loc. cit.* no. 55) might well preclude the acceptance of the combination *B. diversifolia* (Jacq.) Hort. *ex. DC. (loc. cit.* no. 59).

*BIDENS LEPTOLEPIS* Sherff, descript. emend.—Herba annua, erecta, glabra vel fere glabra, ramosa, caule tetragona, circ. 4-6 dm. alta. Folia tenuiter petiolata petiolis 1-3.5 cm. longis, petiolo adjecto usque ad 1.1 dm. longa, pinnatim 3-5 (rariter—7)-partita, foliolis membranaceis, lateralibus plus minusve prorsum spectantibus; omnibus nunc elongato—linearibus et 1-2 mm. latis, vel nunc rursus plus minusve partitis segmentis oblongo—linearibus usque ad 5 mm. latis, ultimis lobis versus apicem rotundatis, apice ipso mucronulatis, margine obsolete vel aegre ciliatis et saepe involutis vel revolutis. Capitula ramos terminantia, pedunculata pedunculis 2-7 vel rarius—15 cm. (supra summum jugum foliorum verorum) longis, radiata, pansa ad anthesin 2.2-3 cm. lata et 0.7-1.1 cm. alta. Involucri bracteae (saepe perspicue) dimorphae, exteriores numerosae (11-18), patentes vel recurvatae, (saepe angustissime) lineari-elongatae, hispidae vel apicem versus glabratae, apice indurato acutae, basi moderate dilatatae, 6-9 mm. longae, interiores ovato-lanceolatae, exterioribus subaequales. Flores ligulati 6-8, ligula oblanceolati vel anguste obovati, valde et perspicue aëropurpurei vel atro-rubri, apice rotundato plus minusve emarginati et saepe 1-5 dentibus minutis denticulati, 0.9-1.5 cm. longi. Achaenia linearia, exalata, infra sensim supra saepe subabrupte angustata, atra, obcompressa, utraque facie circ. 8—sulcata, faciebus marginibusque saepe minute erecto-setosa, corpore 9-14 mm. longa et 1-1.3 mm.

<sup>10</sup> *Fide* Bibl. Franc.: 83. Nov. 29, 1811; *cf.* Jour. Bot. 44:319. 1906.

lata, quam paleae anguste lineares paulo breviora, apice biaristata aristis retrorsum hamosis, circ. 1.7–2 mm. longis.

This species was described originally by me (BOT. GAZ. 76:85, Pl. IX, *figs. a–g*. 1923) from a single specimen by T. Kassner, no. 2725, under trees, Mt. Kundelungu, Belgian Congo, May 10, 1908 (Hb. Berl.).

Shortly afterwards I was able, fortunately, to enlist the friendly cooperation of MR. F. G. OVERLAET of Belgium. In April, 1925, MR. OVERLAET's official duties had taken him to Kafakumba, Belgian Congo, where he collected several handsome specimens of this *Bidens*. These, together with various other Congo specimens found by him, were forwarded to me and are now deposited in certain herbaria (Hb. Field Mus., 3 sheets; Hb. Berl.; Hb. Kew). They afford us a much broader and more representative notion of the species than was possible from the single type specimen. The achenes are mature, the leaves are mostly more divided, with shorter and broader segments, and the external involucre bracts are more often numerous than in the type and also often shorter and less noticeably narrowed. The rays of the two dozen or more flowering heads examined vary from dark red to densely dark purplish-red, as in the type.

With the aid of these additional specimens the above amplified description has been drawn.

*BIDENS TRIPLINERVIA* var. *hirtella* comb. nov.—*B. hirtella* H.B.K. Nov. Gen. et Sp. 4:182 (232). 1820; *B. procumbens* H.B.K. *loc. cit.*; *B. serrata* Pav. ex DC. Prodr. 5:597. 1836.—

The type locality of *B. hirtella* H.B.K. and of *B. procumbens* H.B.K. was not definitely known to KUNTH. In the case of the former, he surmised (H.B.K. *loc. cit.*) that the plant had grown with *B. triplinervia* H.B.K., which was known to have been collected in Mexico ("prope San Augustin de Las Cuevas et urbem Mexici"). In the case of the latter, he surmised that it had grown near Jalapa ("Xalapa"), Mexico. An extra sheet of original material matching the type of *B. hirtella* and of the identical *B. procumbens* was in the Bonpland herbarium, which was given later to the Paris Herbarium (Hb. Mus. Hist. Nat.). This was clearly of the same collection as the two types mentioned. It had been labeled (by Spach, *fide* L. Anfray coram me in mense Augusto, 1914) *B. procumbens*. It came from Quito, Ecuador. Without doubt, then, the types of *B. hirtella* and of *B. procumbens*, which had upon their labels no data at all as to locality (except "Amerique Equatoriale"), came from the same locality.

In a former paper (BOT. GAZ. 80:383. 1925), I have treated *B. triplinervia* as having two sets of forms with divided leaves (var. *mollis* and var. *macrantha*), and one set with undivided leaves (the species proper). From this last set may properly be segregated the forms with slender leaves as var. *hirtella*. KUNTH described the leaves as lanceolate, but they are variously linear-lanceolate, widely linear-oblong, or narrowly oblong-lanceolate. The affinity with *B.*

*tripplinervia* is evidenced by the mostly 5-rayed heads, which alone distinguish the variety from the corresponding variety, next treated, of *B. andicola* H.B.K.

**BIDENS ANDICOLA *tarijensis* var. nov.**—Folia plerumque indivisa, anguste vel late oblongo-linearibus, interdum 1-5-lobata vel 3-5-partita, segmentis linearibus.

*K. Fiebrig* 3460, Tarija, Bolivia, March 9, 1904 (type, Hb. Berl.); *idem* 3461 *pro parte*, alt. 3200 m., Calderillo, Bolivia, March 22, 1904 (Hb. Berl.); *idem* 3004a *pro parte*, Tarija, Bolivia, in 1904 (Hb. Berl.).

**BIDENS ANDICOLA var. *TARIJENSIS* f. *dissecta* f. nov.**—E var. *tarijensi* omnibus foliis pinnatim divisus segmentis elongatis tenuiterque linearibus differt.

*K. Fiebrig* 3147, alt. 2200 m., Tarija, Bolivia, March 13, 1904 (type, Hb. Berl.); *idem* 3004a *pro parte*, Tarija, Bolivia, in 1904 (Hb. Berl.); *idem* 3461 *pro parte*, alt. 3200 m., Calderillo, Bolivia, March 22, 1904 (Hb. Berl.).

The specimens cited here for the var. *tarijensis* and its f. *dissecta* come from the little known territory in the extreme southern part of Bolivia, close to the Argentine boundary. They are graceful, mostly glabrous plants, growing up to a meter in height. They have a habit more characteristic of *B. triplinervia* H.B.K. (with which they were at first confused by me), but the heads are rather uniformly 8-rayed, as in *B. andicola*. The variety proper is seen to be comparable with the var. *hirtella* of *B. triplinervia*. In var. *tarijensis*, however, there is observed the very strong tendency to produce a form with the leaves divided into 3-5 elongate-linear segments. This form (f. *dissecta*) is very different in general habit from other heretofore known forms of *B. andicola* with compound leaves.<sup>11</sup>

**BIDENS PILOSA var. *MINOR* (Bl.) Sherff, loc. cit. 80:387. 1925; *B. africana* Klatt, Bull. Hb. Boiss. 4:464. 1896.**—

In a former article (Sherff, *loc. cit.*) I have given a list of synonyms for the name *B. pilosa* var. *minor*. To those given there, may be added the name *B. africana* Klatt. KLATT's type was a plant collected by Dr. C. Keller, on Reunion Island, summer of 1886. The herbarium containing the type was not cited, nor have I ever succeeded in locating an authentic specimen. At Gray Herbarium, however, are various tracings by KLATT, which form an important part of Klatt's herbarium obtained by that institution some years ago. One of these is of *B. africana*. The plant represented is seen to be widely remote from *B. lantanoides* Gray and *B. hawaiiensis* Gray, with which KLATT compared it in his original

<sup>11</sup> We may note an instructive specimen, *K. Fiebrig* 3459, alt. 2800 m., Tarija, Bolivia, March 22, 1904 (Hb. Berl.). This was collected the same day as the Calderillo plants (no. 3461), at a slightly different altitude in a nearby locality. Its leaves are tripartite and more as in normal forms of *B. andicola*.

description. The tracing, construed together with KLATT's description ("... Die eiförmigen Strahlblüten sind 2-3 mm. lang ..."), shows that the plant itself was, beyond all reasonable doubt, merely a simple-leaved form of *B. pilosa* var. *minor* (Bl.) Sherff.

*BIDENS TRIPARTITA* L. Sp. Pl. 831. 1753; *B. shimadai* Hay. Icon. Pl. Formos. 8:60, fig. 28. 1919.—

Recently HAYATA has interpreted a simple-leaved plant collected by *Y. Shimada* at Shichiseizan, Formosa, September, 1916, as representing a new species, *B. shimadai*. HAYATA describes the plant as near to *B. tripartita*, but states that it differs "in the simple, not lobed, leaves."

The vast literature of *B. tripartita* includes many names proposed by authors, mainly European, to apply to plants of *B. tripartita* that differ in having the leaves simple rather than tripartite. These names must, in the main, be omitted here. It may be noted, however, that as early as 1838 (Petersen. Fl. Lips. 602) a var. *integra* was proposed to embrace the simple-leaved forms. In general, attempts to treat *B. tripartita* and its varieties or forms upon the basis merely of leaf division (without considering also the achenial characters) are as futile as attempts based upon normal, dwarf, and intermediate growth-habit. In neither case does a natural arrangement result.

While I have not seen the *Shimadai* specimen, nor even any specimen of *B. tripartita* from Formosa,<sup>12</sup> I have examined a considerable number of specimens from Japan proper and from Corea. Among these were several forms which matched the HAYATA description more or less, as to leaves, but were still too close to *B. tripartita* proper to permit segregation.<sup>13</sup>

<sup>12</sup> HAYATA lists, however, a normal specimen of *B. tripartita* from the same locality in Formosa—*T. Soma*, Shichiseizan, March, 1916. He states that *B. tripartita* is new to the flora of Formosa.

<sup>13</sup> Several additional specimens from the same range were found to have the achenes more weakly setose, or even glabrous, upon the margins, and to be referable to *B. tripartita* var. *repens* (D. Don) Sherff.

A complete list of specimens of *B. tripartita* from Corea and Japan, examined by me, follows: *Anon.*, Sakawa, Shikoku Island, Japan, October 10, 1890 (Hb. Berl.); *Abbé U. Faurie* 162, Shonai, Japan, September 22, 1897 (Hb. Berl.; Hb. Univ. Vienna); *idem* 417, Fusan, Corea, October 4, 1901 (Hb. Berl.) *idem* 1171, Mombetsu, Japan, September 27, 1887 (Hb. Kew, 2 sheets); *idem* 3372, Aomori, Japan, October, 1899 (Hb. Berl.); *idem* 4854 and 4855, Kushiro, Japan, September 19, 1889 (Hb. Kew); *Maximowicz*, Hakodate, Japan, 1861 (Hb. Univ. Copenh.; Hb. Mus. Vienna); *idem*, Yokohama, Japan, 1862 (Hb. Berl.); *K. Menjabe*, Azuma, Prov. Iwuri, Japan, August 22, 1884 (Hb. Gray); *J. Natsumura*, Tokyo, Japan, September 30, 1879 (Hb. Univ. Vienna); *K. Saida*, Omiya near Tokyo, Japan, July, 1885 (Hb. Berl.); *Y. Tanaka* 20, Japan (Hb. Mus. Vienna); *Taquet* 1028, Hongno, Corea, October 15, 1908 (Hb. Berl.; Hb. Deless.); *Tokubuchi*, Horomambetsu, Prov. Hidaka, Japan, August 20, 1892 (Hb. Mo. Bot. Gard.); *K. Watanabe*, Sakawa, Shikoku Island, Japan, October 11, 1888 (Hb. Gray).

*BIDENS ANGUSTISSIMA* var. *linifolia* (Schz. Bip. ex Klatt) Sherff, BOT. GAZ. 81:49. 1926; *Coreopsis linearifolia* DC. Prodr. 5:570. 1836; *Bidens linifolia* Schz. Bip. ex Klatt, Flora 68:203. 1885.—

DE CANDOLLE's type of *Coreopsis linearifolia* was collected by *Keerl* at Tlapucoya (Tlapujahua), Puebla, Mexico. I myself have not seen DE CANDOLLE's material ([ex Hb. Mart. in] Hb. Deless.), but am indebted to Dr. S. F. BLAKE, Associate Botanist, United States Bureau of Plant Industry, for critical notes upon it. Dr. BLAKE examined *Keerl*'s plant recently and reported (*in litt.* Jan. 5, 1926) that it belonged to *B. angustissima* H.B.K. From DECANDOLLE's description and his placement of it among the species with leaves undivided, it is seen at once that the plant is of the var. *linifolia*. We may note in passing that many beautiful specimens of this same variety were collected by *Pringle* not far to the west of Tlapucoya (*Pringle* 6924, alt. 9500 ft., Cerro Ventoso above Pachuca, Hidalgo, Mexico, August 18, 1898;—Hb. Berl.; Hb. Boiss.; Hb. Carn. Mus.; Hb. Deless.; Hb. Field Mus.; Hb. Kew; Hb. Mo. Bot. Gard.; Hb. Mun.; Hb. N.Y. Bot. Gard.; Hb. Par.; Hb. Philad.; Hb. Mus. Vienna; Hb. Univ. Vienna, etc.).<sup>14</sup>

*BIDENS AUREA* var. *wrightii* (Gray) comb. nov.; *B. heterophylla* var. *wrightii* Gray, Proc. Amer. Acad. 19:15. 1883; Synopt. Fl. N. Amer. 1<sup>st</sup>:298. 1884.—

In a former article (BOT. GAZ. 81:42. 1926), I have shown that the name *B. aurea* (Ait.) Sherff must stand for the plants long known to authors under the synonym *B. heterophylla* Ort. It may be noted here that one of the many forms of this species has seemed distinct enough to various students to warrant maintenance separately as var. *wrightii*. For those who accept such a status the name becomes *B. aurea* var. *wrightii*.

*BIDENS SCHIMPERI* Schz. Bip. ex Walpers Repert. 6:168. 1846; *B. proluxa* S. L. Moore, Jour. Proc. Linn. Soc. 40:116. 1911; *B. punctata* Sherff, BOT. GAZ. 59:302. 1915. —

I have already called attention (BOT. GAZ. 81:53. 1926) to the "amazing range of variation in all respects" manifested by *B. schimper* Schz. Bip. *B. proluxa* Moore was founded upon *C. F. M. Swynnerton* 1884, northern Melssetter at alt. 200-600 ft., Rhodesia, April, 1907 (Hb. Brit. Mus.). MOORE (*loc. cit.*) distinguished this plant from *B. schimper* by "its slender habit with long stalked scattered heads, its smaller leaves with narrower lobes, its smaller heads, shorter involucre and narrower ligules." Upon comparison with a considerable number of specimens of *B. schimper*, however, *B. proluxa* is found to be connected much too closely for specific separation.

<sup>14</sup> BLAKE had compared the *Pringle* plant with *Keerl*'s plant. He stated (*in litt.* Jan. 26, 1926) that "*Pringle* 6924 was a perfect match."

*B. punctata* Sherff was founded upon two sheets of material (Hb. Kew) consisting of small plants with leaves tripartite or even entire, not bi- or tripinnate as in ordinary *B. schimperi*. Their outer involucre bracts were noticeably shorter than the inner ones. The fruiting characters were very similar to those of *B. prolixa*, and I therefore came later to regard *B. punctata* and with it the less extreme *B. prolixa* as representing a variety (var. *punctata* comb. adhuc ined.) of *B. schimperi*. The diagnostic characters are found in herbarium specimens, however, to be so uncorrelated and so prone to appear in different combinations, that it seems difficult to justify maintenance even of varietal rank for the *prolixa* or *punctata* forms.

**BIDENS SPECIES NATIVE TO ISLANDS OF CENTRAL PACIFIC OCEAN.**—In the preparation of a monograph of the entire genus *Bidens*, I have recently completed a conspectus of all the known species and varieties of *Bidens* which are native to the various islands of the central Pacific. This conspectus, together with an analytical key to the species and varieties, is here published separately from the proposed monograph, with the intention of making the results of my studies more immediately useful to various plant workers in the region included:<sup>15</sup>

**Key to species and varieties of *Bidens* native to islands of  
central Pacific Ocean<sup>16</sup>**

- a. Leaves commonly undivided:
  - b. Inflorescence composed of solitary, pedunculate head:
    - c. Leaves tomentose-pubescent. . . . . 6. *B. lantanoides*
    - c. Leaves glabrous:
      - d. Petioles equaling or surpassing blades. . . 33. *B. mauianensis*
      - d. Petioles shorter than blades:
        - e. Leaves broadly cuneate at base, on each lateral margin coarsely dentate with 3–5 teeth. . . . . 35. *B. cuneata*
        - e. Leaves rounded or cordate at base, on each lateral margin serrate with 6–15 teeth. . . . . 34. *B. molo-kaiensis*
  - b. Inflorescence composed of few or many heads, cymose or paniculate-corymbose:

<sup>15</sup> Introduced species such as *B. laevis* (L.) B. S. P., *B. pilosa* L., *B. chinensis* (L.) Willd., and *B. tripartita* L. are omitted.

<sup>16</sup> As here taken, extending from the Tropic of Cancer south to slightly past the Tropic of Capricorn (Pitcairn Isl.) and from 130° to 180° W. Longitude.

- c. Heads at anthesis minute, 2-4 mm. high:
  - d. Achenes 6-8 mm. long; species of Hawaiian Islands
    - 11. *B. degeneri*
  - d. Achenes commonly 2.5-6 mm. long; species of Southern Hemisphere:
    - e. Leaves more or less oblong-ovate, shortly acuminate, their thickish petioles 1.2-1.4 mm. wide . . . 1. *B. ahnnei*
    - c. Leaves lanceolate-ovate, cordate-acuminate, their slender petioles 0.6-0.8 mm. wide . . . . . 2. *B. polycephala*
    - c. Leaves narrower, as a rule narrowly lanceolate or oblong-lanceolate . . . . . 7. *B. australis*
- c. Heads at anthesis larger:
  - d. Leaves as a rule narrowly lanceolate or oblong-lanceolate:
    - e. Plants of Marquis Islands:
      - f. Achenes about 4 mm. long . . . . . 3. *B. jardinii*
      - f. Achenes about 6 mm. long . . . . . 4. *B. bipontina*
    - e. Plants not known from Marquis Islands (nor from Hawaiian Islands)
      - f. Heads few ( $\pm 8$  on a single branch) . . 8. *B. mathewsii*
      - f. Heads more numerous . . . . . 7. *B. australis*
  - d. Leaves broader:
    - c. Leaves pubescent underneath . . . . . 5. *B. cordifolia*
    - c. Leaves glabrous:<sup>17</sup>
      - f. Stems or branches more or less glaucous; plants of Hawaiian Islands:
        - g. Exterior involucre bracts 1-2.5 mm. long:
          - h. Simple leaves ovate or rhomboid-ovate
            - 15. *B. ctenophylla*
          - h. Simple leaves oblong-lanceolate
            - 10. *B. hawaiiensis*
        - g. Exterior involucre bracts about 4-5 mm. long
          - 12. *B. asymmetrica*
      - f. Neither stems nor branches glaucous:
        - g. Leaves ovate-lanceolate, cuneate at base, petioles 1-3 cm. long. Plant known only from Island of Nukahiva (Marquis Isls.) . . . . . 9. *B. henryi*

<sup>17</sup> Low, bushy, dwarfed forms of *B. graciloides* with simple leaves may be looked for here.

- g. Leaves ovate, subcordate at base; petioles 2-5 cm. long. Plant known only from Island of Oahu (Hawaiian Isls.) . . 16. *B. macrocarpa* var. *ovatifolia*
- a. Leaves commonly divided:
  - b. Heads large, at anthesis 5 cm. wide; styles surpassing the anthers by 6-10 mm., their branches abruptly long-caudate  
36. *B. cosmoides*
  - b. Heads smaller; usually only stylar branches projecting above the anthers:
  - c. Leaves bipinnately divided, their ultimate segments narrow (or in *B. waianensis* sometimes broad):
  - d. Plants decumbent or ascending:
    - e. Heads solitary or long-pedunculate  
33. *B. mauiensis* and var. *lanaiensis*
    - e. Heads corymbose or paniculate. . . 32. *B. hillebrandiana*
  - d. Plants erect:
    - e. Achenes spirally coiled or twisted; segments of leaves broadly linear or wider. . . . . 31. *B. waianensis*
    - e. Achenes straight or slightly twisted, 8-12 mm. long, segments of leaves linear or filiform, or if broader then with conspicuously elongate, slender teeth  
30. *B. menziesii* and vars. *filiformis* and *leptodonta*
    - c. Achenes straight or nearly so, about 3 mm. long  
19. *B. pulchella*
- c. Leaves ternately or pinnately divided, or sometimes imperfectly bipinnate:
- d. Plants decumbent or ascending:
  - e. Heads solitary, long-pedunculate. . . . 33. *B. mauiensis*
  - e. Heads cymose, corymbose or paniculate:
    - f. Paleae up to 1 cm. long, exceeding the mature, biaristate achenes. . . . . 32. *B. hillebrandiana*
    - f. Paleae much shorter and much surpassed by the usually exaristate achenes  
11. *B. degeneri* var. *apioides*
- d. Plants erect:
  - e. Principal achenial aristac, unless absent, commonly arising from below apex of achene and continuous with achene's margins:



- f. Achenes narrow (0.5–1.5 mm. wide), black, wingless; heads at anthesis 4–6 mm. high and 1.5–2 cm. wide:
- g. Heads numerous, often densely corymbose or paniculate; mature achenes commonly glabrous upon the face and margins:
- h. Mature achenes conspicuously and intensely shining-black; exterior involucre bracts only about 1.5 mm. long
- 29. *B. micrantha* and vars. *kaalana* and *laciniata*
- h. Mature achenes somewhat shiny and becoming black, but not intensely so; exterior involucre bracts larger, mostly 3–5 mm. long
- 21. *B. conjuncta*
- g. Heads fewer, cymose-corymbose or scattered here and there, mature achenes dull black, setose upon the margins.....28. *B. waimeana*
- f. Achenes broader, brownish, often winged; heads at anthesis 7–8 mm. high and about 3 cm. wide
- 16. *B. macrocarpa*
- c. Achenial aristae missing or principal ones commonly arising from achenial apex itself:
- f. Outer involucre of immature heads reflexed; inner involucre conspicuous and similar to that of *Cosmos bipinnata* Cav. ....27. *B. amplexans*
- f. Exterior bracts of immature involucre erect or spreading:
- g. Heads commonly solitary, peduncles up to 11 cm. long, exterior bracts notably foliaceous
- 25. *B. valida*
- g. Heads and exterior bracts otherwise:
- h. Achenes spirally coiled or strongly twisted:
- i. Achenes commonly coiled through 3–5 revolutions.....23. *B. torta*
- i. Achenes commonly coiled through not more than 2 revolutions:
- j. Cauline leaves large, 3-parted:
- k. Flowering heads numerous, small, 4–5 mm. high and about 1.5 cm. wide

- 22. *B. forbesii*
- k. Flowering heads few, larger, 6–12 mm. high and  $\pm 2$  cm. wide
- 24. *B. campylotheca*
- j. Cauline leaves commonly 5–7 parted:
  - k. Their lower leaflets often alternate; achenes narrowly linear, toward apex narrowly elongate. . . . . 13. *B. cervicata*
  - k. Their lower leaflets regularly opposite; achenes only slightly narrowed above:
    - l. Internodes long; leaves few and large; heads few, when expanded 3–4 cm. wide
    - 24. *B. campylotheca* var. *pentamera*
    - l. Internodes short; leaves usually rather numerous, of medium size; heads usually very numerous, single herbarium specimen bearing 50–200, these when expanded only about 1.5–2 cm. wide
- 31. *B. waianensis*
- h. Achenes straight, curved or only weakly twisted:
  - i. Branches of inflorescence widely spreading, bearing 1–few heads; internodes of stem and branches commonly long
  - 24. *B. campylotheca* and vars. *nematocera* and *pentamera*
- i. Habit otherwise:
- j. Leaves commonly 3-parted:
  - k. Leaflets narrowly lanceolate, crenate-serrate, terminal one long-attenuate at apex; heads not numerous
  - 18. *B. asplenioides*
  - k. Leaflets lanceolate to ovate (or, if more elongate, then heads numerous), acutely serrate, terminal one apically acute or acuminate but not long-attenuate:
    - l. Flowering heads minute, 6–8 mm. wide and 4–5 mm. high

- 11. *B. degeneri* var. *apioides*
  - l. Flowering heads larger:
    - m. Stem and branches acutely tetragonal, upwardly herbaceous:
    - n. Lateral leaflets usually sessile; body of achene 10-16.5 mm. long
  - 21. *B. conjuncta*
    - n. Lateral leaflets petiolulate; body of achene 6-9 mm. long
  - 20. *B. sandvicensis* and var. *caduca*
    - m. Stem and branches rounded-tetragonal, scarcely herbaceous above:
    - n. Mature achenes more or less twisted; exterior involucre bracts shorter by half than interior
  - 12. *B. asymmetrica*
    - n. Mature achenes straight or curved, not twisted:
    - o. Exterior involucre bracts commonly almost, or sometimes fully equaling interior ones
  - 28. *B. waimeana*
    - o. Exterior involucre bracts shorter than interior ones by third or half. . . . . 14. *B. graciloides*
- j. Leaves more often 5-parted;
  - k. Heads in clusters of 2-5, on long, slender, erect, naked branches, separately pedunculate on peduncles mostly 2-5.5 cm. long; at anthesis 7-8 mm. high
  - 26. *B. stokesii*
    - k. Heads cymose-corymbose or paniculate, with peduncles mostly shorter at anthesis, 4.5-7 mm. high:
    - l. Achenes glabrous or sparsely setose above:

- m. Leaflets narrowly lanceolate, crenate-serrate, terminal one long-attenuate at apex; herb found on the Island of Niihau (Hawaiian Isls.)
  - 18. *B. asplenioides*
- m. Leaflets lanceolate to ovate, acutely serrate, terminal one apically acute or acuminate but not long-attenuate
  - 20. *B. sandvicensis*
- l. Achenes moderately or copiously setose upon margins:
  - m. Plants commonly 2–5 dm. high:
    - n. Inflorescence manifestly exerted above leaves
      - 17. *B. micranthoides*
    - n. Inflorescence (on plant as a whole) not manifestly exerted above leaves
      - 14. *B. graciloides*
  - m. Plants commonly 4–9 dm. high:
    - n. In habit approaching *B. sandvicensis*; terminal leaflet of a larger leaf having in all about 22–26 teeth
      - 20. *B. sandvicensis* var. *setosa*
    - n. In habit approaching *B. asymmetrica*; terminal leaflet of a larger leaf having in all about 6–16 teeth
      - 28. *B. waimeana*

**Conspectus of species and varieties of *Bidens* native to  
islands of central Pacific Ocean<sup>18</sup>**

1. *B. AHNNEI* Sherff, BOT. GAZ. 76:165. 1923. (PL. I, figs. *i-p*)—Isl. of Nukahiva (Marquis Isls.).
2. *B. POLYCEPHALA* Schz. Bip., Flora 39:360. 1856; *non* Oerst. ex Fiek Fl. Schlesien 218. 1881; *Campylothecha polycephala* Schz. Bip.

<sup>18</sup> Cf. footnote 16.

*loc. cit.*; *Coreopsis polycephala* (Schz. Bip.) Benth. and Hook *ex* Drake del Cast. Illustr. Fl. Ins. Mar. Pacif. 210. 1890; *cf.* Fl. Polynes. Fr. 108. 1892. (PL. I, figs. *a-h*)—Isl. of Nukahiva (Marquis Isls.).

3. B. JARDINII Schz. Bip., *loc. cit.*; *Campylotheca jardinii* Schz. Bip. *loc. cit.*; *Coreopsis jardinii* (Schz. Bip.) Drake del Cast.—Isl. of Nukahiva (Marquis Isls.).

4. B. BIPONTINA Sherff, *supra* p. 10. *B. serrulata* Schz. Bip. *loc. cit.*; *non B. serrulata* (Poir.) Desf. Tabl. Ecol. Bot. Edit. II: 130. 1815. *Campylotheca serrulata* Schz. Bip. *loc. cit.*; *Coreopsis serrulata* (Schz. Bip.) Benth. and Hook. *ex* Drake del Cast. *loc. cit.*—Isl. of Nukahiva (Marquis Isls.).

5. B. CORDIFOLIA Schz. Bip., Flora 39:361. 1856; *Campylotheca cordifolia* Schz. Bip. *loc. cit.*; *Coreopsis cordifolia* (Schz. Bip.) Drake del Cast. Illustr. Fl. Ins. Mar. Pacif. 208. 1890.—(PL. v, figs. *h-n*)—Isl. of Nukahiva (Marquis Isls.).

6. B. LANTANOIDES Gray, Proc. Amer. Acad. 5:128. 1861. (PL. v, figs. *a-g*)—Isles of Eimeo<sup>19</sup> and Tahiti (Society Isls.).

7. B. AUSTRALIS Spreng. Syst. 3:453. 1826; *Bidens australis* Spreng. *in* Sherff, BOT. GAZ. 81:32. 1926;—*Coreopsis fruticosa* Forst. Prodr. Fl. Ins. Austr. 91. 1786 (*nomen; non* Vest); *Campylotheca australis* (Spreng.) Less., Linnæa 6:509. 1831; *Bidens paniculata* Hook. and Arn. Bot. Beech. Voy. 66. 1841; *B. fruticosa* Schz. Bip., Flora 39:358. 1856 (*non* L., *non* DC.); *Coreopsis fruticosa* Solander *mss.*, *in* Seemann Fl. Vitiensis 143. 1865 1868.—Isles of Eimeo<sup>20</sup> and Tahiti (Society Isls.) and also Tonga Isls.<sup>21</sup>

8. B. MATHEWSII Sherff, *loc. cit.* 81:34. 1926.—Pitcairn Isl.

9. B. HENRYI Sherff, *loc. cit.* 76:164. 1923. (PL. II, figs. *a-i*)—Isl. of Hiva-Oa (Marquis Isls.).

10. B. HAWAIIENSIS Gray, Proc. Amer. Acad. 5:128. 1861; *Campylotheca hawaiiensis* Hillebr. Fl. Hawaii. Isls. 211, 214. 1888; *Coreopsis hawaiiensis* (Gray) Drake del Cast. Illustr. Fl. Ins. Mar. Pacif. 209. 1890.—Isles of Hawaii and Lanai (Hawaiian Isls.).<sup>22</sup>

11. B. DEGENERI Sherff, *supra* p. 3; *Campylotheca dichotoma*

<sup>19</sup> Known also as Morea.

<sup>20</sup> Cf. footnote 19.

<sup>21</sup> Known also as Friendly Isls.

<sup>22</sup> Also in Eastern Maui, *vide* Hillebr. *loc. cit.* 214.

Hillebrand Fl. Hawaii. Isls. 211, 212. 1888; *Bidens dichotoma* (Hillebr.) Sherff, *loc. cit.* 70:98. 1920 (*non* *B. dichotoma* Desf. Tabl. Edit. I:108. 1804 [*—nomen nudum*]; *et* Cat. Pl. Hort. Reg. Paris. Edit. III:185. 1829).—Isls. of Oahu, Molokai and Maui (Hawaiian Isls.).

12. *B. ASYMMETRICA* (Lévl.) Sherff, *loc. cit.* 81:43. 1926; *Bidens gracilis* Nutt., Trans. Amer. Phil. Soc. Ser. II. 7:368. 1841; Sherff, *loc. cit.* 70:105 and Pl. XIII. 1920; *non* *B. gracilis* Torr. in Ann. Lyc. N.Y. 2:215. 1828; *Campylothea gracilis* (Nutt.) Walp. Repert. Bot. Syst. 2:618. 1843; *Lipochaeta asymetrica* Lévl., Fedde Repert. Spec. Nov. 10:122. 1911. (PL. III.)—Isl. of Oahu (Hawaiian Isls.).

13. *B. CERVICATA* Sherff, *loc. cit.* 70:99.—1920.—Isl. of Kauai (Hawaiian Isls.).

14. *B. GRACILOIDES* Sherff, *loc. cit.* 76:159. 1923. (PL. IV.)—Isl. of Oahu (Hawaiian Isls.).

15. *B. CTENOPHYLLA* Sherff, *supra* p. 5.—Isl. of Hawaii (Hawaiian Isls.).

16. *B. MACROCARPA* (Gray) Sherff, *loc. cit.* 70:97. 1920; *Coreopsis* (*Campylothea*) *macrocarpa* Gray, Proc. Amer. Acad. 5:126. 1861.—Isl. of Oahu (Hawaiian Isls.).

*B. MACROCARPA* var. *OVATIFOLIA* (Gray) Sherff *supra* p. 7; *B. sandwicensis* var. *ovatifolia* Gray, Proc. Amer. Acad. 5:128. 1861; *Campylothea macrocarpa* var. *ovatifolia* (Gray) Hillebrand Fl. Hawaii. Isls. 215. 1888.—Isl. of Oahu (Hawaiian Isls.).

17. *B. MICRANTHOIDES* Sherff, *loc. cit.* 70:100. 1920; *B. angustifolia* Nutt., Trans. Amer. Phil. Soc. Ser. II. 7:369. 1841; *non* *B. angustifolia* Lam. Encycl. 1:416. 1789; *Campylothea angustifolia* (Nutt.) Walp. Repert. 2:618. 1843.—Isls. of Kauai and Oahu (Hawaiian Isls.).

18. *B. ASPLENIOIDES* Sherff, *loc. cit.* 70:101 and Pl. XII, figs. a-f. 1920.—Isl. of Niihau (Hawaiian Isls.).

19. *B. PULCHELLA* (Less.) Schz. Bip., Flora 39:357. 1856; *Adenolepis pulchella* Less., Linnaea 6:510, tab. 6. 1831; *Campylothea pulchella* (Less.) Hillebrand Fl. Hawaii. Isls. 211, 212. 1888; *Coreopsis pulchella* (Less.) Drake del Cast. Illustr. Fl. Ins. Mar. Pacif. 210. 1890. (PL. II, figs. j-p)—Isl. of Oahu (Hawaiian Isls.).

20. *B. SANDVICENSIS* Less., *Linnaea* 6:508. 1831; Sherff, *loc. cit.* 76:161. 1923; *B. mutica* Nutt., *Trans. Amer. Phil. Soc. Ser. II.* 7:368. 1834; *Campylothea mutica* (Nutt.) Walp. *Repert.* 2:618. 1843; *C. sandvicensis* (Less.) Hillebr. *Fl. Hawaii. Isls.* 211, 214. 1888; *C. macrocarpa* var.  $\gamma$  Hillebr. *loc. cit.* 215; *Coreopsis sandvicensis* (Less.) Benth. and Hook *ex* Drake del Cast. *Illustr. Fl. Ins. Mar. Pacif.* 210. 1890.—Isles of Kauai, Oahu, Maui, and Hawaii (Hawaiian Isles.).

*B. SANDVICENSIS* var. *CADUCA* Sherff, *supra* p. 7.—Isl. of Molokai (Hawaiian Isles.).

*B. SANDVICENSIS* var. *SETOSA* Sherff *supra* p. 6; *B. setosa* Sherff, *loc. cit.* 70:103. 1920.—Isles of Kauai and Hawaii (Hawaiian Isles.).<sup>23</sup>

21. *B. CONJUNCTA* Sherff, *BOT. GAZ.* 76:162. 1923.—Isles of Oahu and Maui (Hawaiian Isles.).—Perhaps only a variety of *B. sandvicensis*.

22. *B. FORBESII* Sherff, *loc. cit.* 70:103 and Pl. XIV. 1920.—Isl. of Kauai (Hawaiian Isles.).

23. *B. TORTA* Sherff, *loc. cit.* 70:105. 1920.—Isl. of Oahu (Hawaiian Isles.).

24. *B. CAMPYLOTHECA* Schz. Bip., *Flora* 39:359. 1856; *Campylothea grandiflora* DC. *Prodr.* 5:593. 1836; *Coreopsis macraei* Gray, *Proc. Amer. Acad.* 5:126. 1861.—Isles of Oahu, Lanai, and Hawaii (Hawaiian Isles.).

*B. CAMPYLOTHECA* var. *NEMATOCERA* Sherff, *supra* p. 7.—Isl. of "Molokai and probably also of Maui" (Hawaiian Isles.).<sup>24</sup>

*B. CAMPYLOTHECA* var. *PENTAMERA* Sherff, *supra* p. 4.—Isl. of Maui (Hawaiian Isles.).

<sup>23</sup> *B. sandvicensis* var. *heterophylla* Gray (*Proc. Amer. Acad.* 5:128. 1861) was described supposedly from Captain Beechey's plant collected on the Island of Oahu, and determined by HOOKER and ARNOTT as *Bidens luxurians* Willd. This specimen is still at Kew in good condition but with capitula too immature for satisfactory determination. The plant is probably a mere growth-form of *B. sandvicensis* Less. GRAY cited "*B. luxurians* Hook. and Arn." as a synonym, but complicated his variety's status by citing material collected by the *U.S. South Pacific Exploring Expedition* under Captain Wilkes. At Gray Herbarium I did not find this, but I did find *J. Remy* 281, labeled by GRAY "*B. sandvicensis* var. *heterophylla*." This last plant (*cf.* *BOT. GAZ.* 70:97, footnote 9. 1920) is referable to *B. micrantha* Gaud.

<sup>24</sup> *Fide* HILLEBRAND, *loc. cit.* 215.

25. *B. VALIDA* Sherff, *loc. cit.* 70:102. 1920.—Isl. of Kauai (Hawaiian Isls.).

26. *B. STOKESII* Sherff, *loc. cit.* 70:101 and Pl. XII, figs. 8-9. 1920.—Isl. of Niihau (Hawaiian Isls.).

27. *B. AMPLECTENS* Sherff, *loc. cit.* 70:99. 1920.—Isl. of Oahu (Hawaiian Isls.).

28. *B. WIAMEANA* Sherff, *loc. cit.* 76:164. 1923.—Isl. of Kauai (Hawaiian Isls.).

29. *B. MICRANTHA* Gaud., Voy. Freycinet Bot. Pl. 85 (without description). 1826; *ibid.* 464. 1829; *Campylothea micrantha* (Gaud.) Cassini, Dict. Sc. 51:475. 1827; *Coreopsis micrantha* (Gaud.) Gray, Proc. Amer. Acad. 5:127. 1861; *B. sandwicensis* var. *heterophylla* Gray (excl. syn. *B. luxurians* Hook. and Arn.), *loc. cit.* 128; *B. remyi* Drake del Cast. Illustr. Fl. Ins. Mar. Pacif. 39. 1888 (*non B. remyi* [Hillebr.] Sherff); *Coreopsis remyi* Drake del Cast. *loc. cit.* 210. 1890.—Isles. of Oahu,<sup>25</sup> Lanai, Maui, and Hawaii (Hawaiian Isls.).

*B. MICRANTHA* var. *LACINIATA* (Hillebr.) Sherff, *supra* p. 8; *Campylothea micrantha* var. *laciniata* Hillebrand Fl. Hawaii. Isls. 216. 1888.—Isl. of Maui (Hawaiian Isls.).

*B. MICRANTHA* var. *KAALANA* Sherff, *supra* p. 8; *Campylothea micrantha* var.  $\gamma$  Hillebrand, *loc. cit.*—Isl. of Oahu (Hawaiian Isls.).

30. *B. MENZIESII* (Gray) Sherff, *loc. cit.* 70:98. 1920; *Coreopsis* (*Campylothea*) *menziesii* Gray, Proc. Amer. Acad. 5:127. 1861.—Isles. of Molokai, Maui, and Hawaii (Hawaiian Isls.). The plants on Maui and Hawaii tend strongly to pass over into the following varieties:

*B. MENZIESII* var. *LEPTODONTA* Sherff, *supra* p. 9; *Campylothea menziesii* var.  $\beta$  Hillebrand Fl. Hawaii. Isls. 216. 1888.—Isles. of Maui and Hawaii (Hawaiian Isls.).

<sup>25</sup> Only one collection is known to me from Oahu, viz. *Ad. von Chamisso*, in 1817. My manuscript cites Hb. Univ. Halle, Hb. Kew and Hb. Petrop. for the Chamisso specimens. I had noted the two specimens at Leningrad (Hb. Petrop.) as being a "forma foliis indivisis." The Kew sheet, now before me, bears several very short branches or ends of branches, with small, simple leaves. These leaves have the thickish texture common to normal *B. micrantha* leaves and do not appear to represent the var. *kaalana*, which variety is the only representative of the species collected upon Oahu for more than a century.



B. MENZIESII var. FILIFORMIS Sherff, *supra* p. 9:  
*Campylothea menziesii* var.  $\gamma$  Hillebrand *loc. cit.*—Isl. of  
Hawaii (Hawaiian Isls.).

31. B. WAIANENSIS Sherff, *loc. cit.* 70:104. 1920.—Isl. of Oahu  
(Hawaiian Isls.).

32. B. HILLEBRANDIANA (Dr. del Cast.) Degener, *supra* p. 6;  
*Campylothea remyi* Hillebrand Fl. Hawaii. Isls. 211, 212. 1888;  
*Coreopsis hillebrandiana* Drake del Cast. Illustr. Fl. Ins. Mar. Pacif.  
209. 1890; *Bidens remyi* (Hillebr.) Sherff, *loc. cit.* 70:97. 1920 (*non*  
*B. remyi* Drake del Cast. Illustr. Fl. Mar. Pacif. 39. 1888); *Cam-*  
*pylothea rufifolia* Lévl., Fedde Repert. Spec. Nov. 10:123. 1911.—  
Isl. of Molokai, Maui, and Hawaii (Hawaiian Isls.).

33. B. MAUIENSIS (Gray) Sherff, *loc. cit.* 70:98. 1920; *Coreopsis*  
*mauiensis* Gray. Proc. Amer. Acad. 5:125. 1861; *Campylothea*  
*mauiensis* (Gray) Hillebrand Fl. Hawaii. Isls. 211, 213. 1888.—Isl.  
of Maui (Hawaiian Isls.).

B. MAUIENSIS var. LANAIENSIS Sherff, *loc. cit.* 80:381.  
1925; *Campylothea mauiensis* var.  $\beta$  Hillebrand Fl.  
Hawaii. Isls. 213. 1888.—Isls. of Lanai and Maui  
(Hawaiian Isls.).

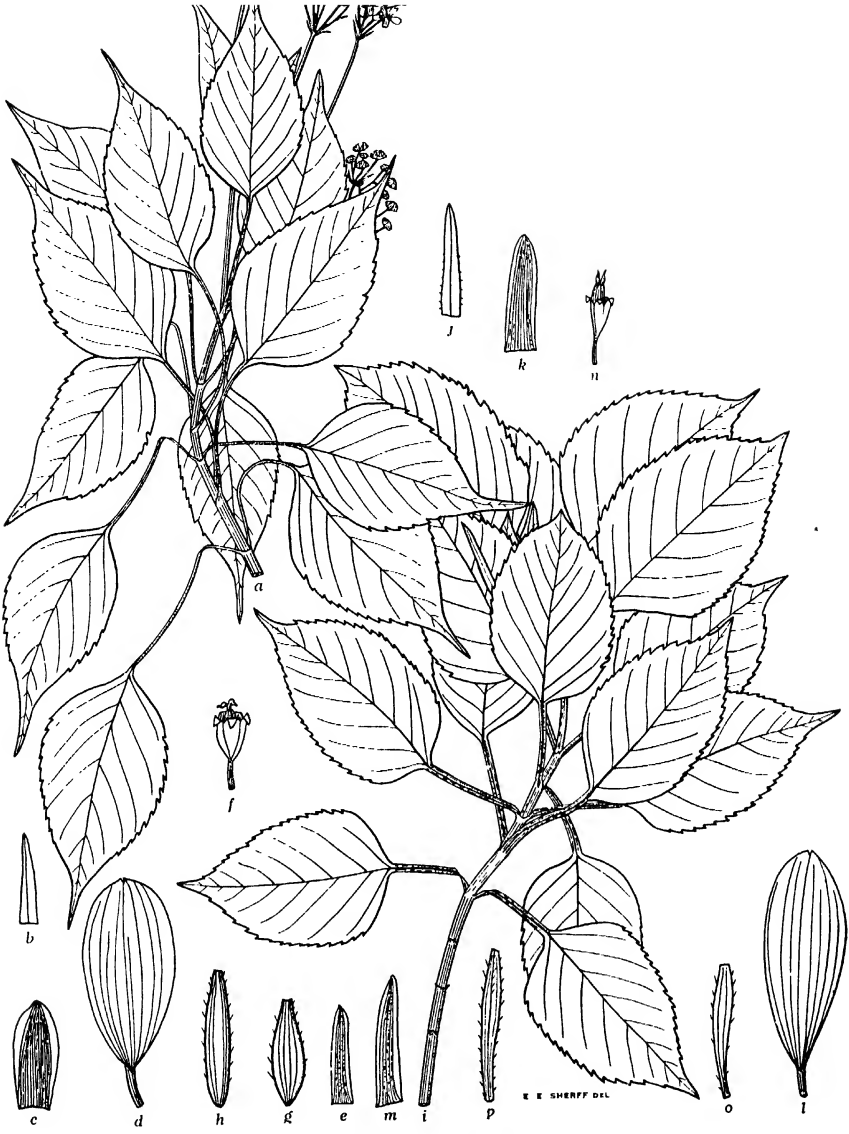
34. B. MOLOKAIENSIS (Hillebr.) Sherff, *loc. cit.* 70:97. 1920;  
*Campylothea molokaiensis* Hillebrand Fl. Hawaii. Isls. 211, 212.  
1888; *Coreopsis molokaiensis* (Hillebr.) Drake del Cast. Illustr. Fl.  
Ins. Mar. Pacif. 210. 1890.—Isl. of Molokai (Hawaiian Isls.).

35. B. CUNEATA Sherff, *loc. cit.* 70:102 and Pl. XIII. 1920.—Isl.  
of Oahu (Hawaiian Isls.).

36. B. COSMOIDES (Gray) Sherff, *loc. cit.* 70:98. 1920; *Coreopsis*  
(*Campylothea*) *cosmoides* Gray, Proc. Amer. Acad. 5:126. 1861.—  
Isl. of Kauai<sup>26</sup> (Hawaiian Isls.).

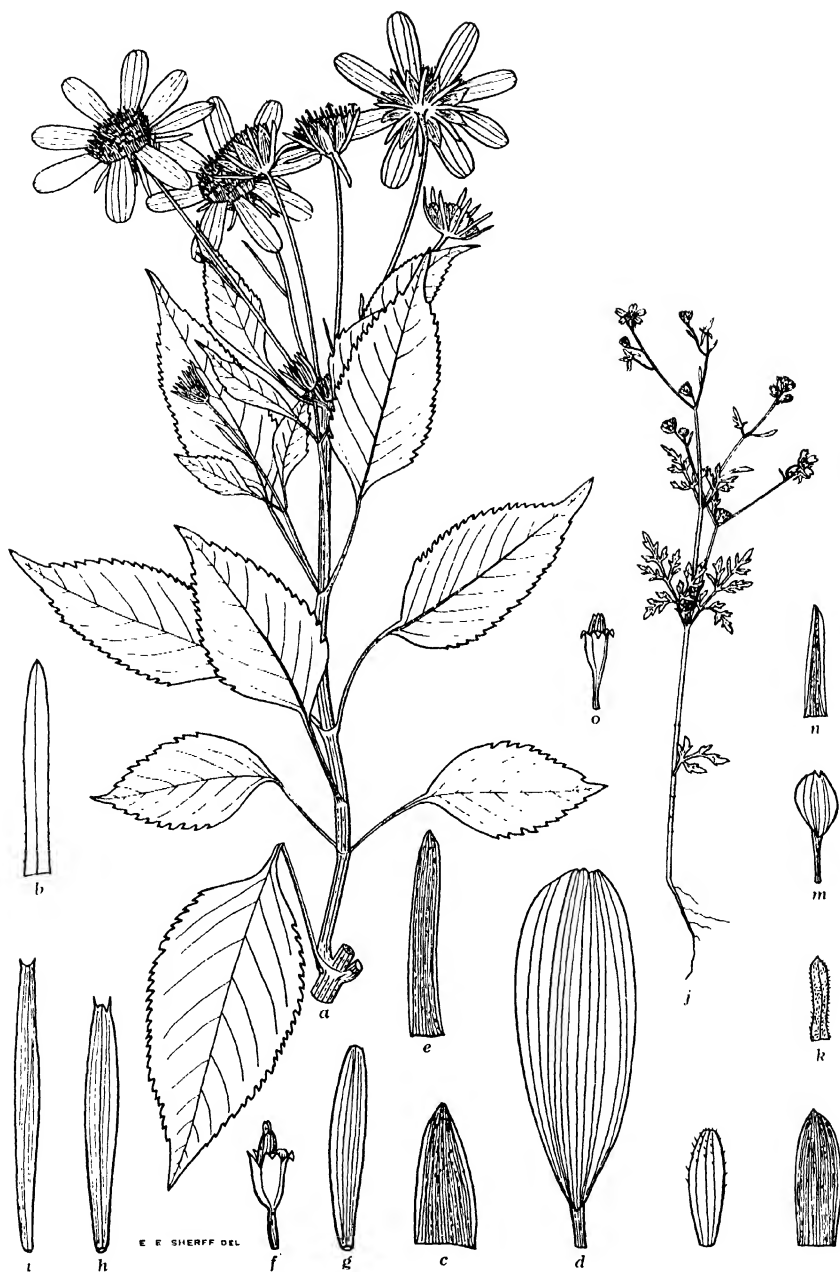
CHICAGO NORMAL COLLEGE

<sup>26</sup> A sheet in Hb. Kew (*Mrs. Francis Sinclair Jr.*) has "Hawaii" on label. It may be, however, that the Hawaiian Archipelago was meant, rather than the Isl. of Hawaii itself.



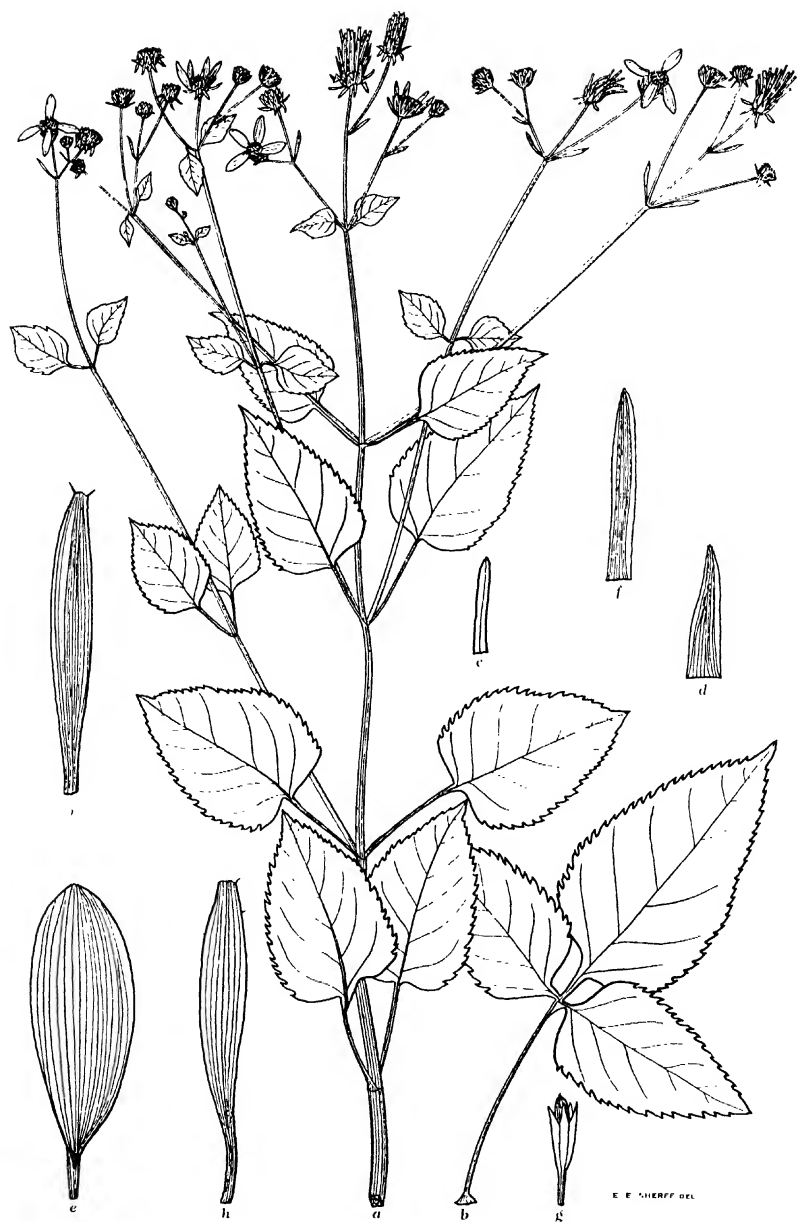
SHERFF on BIDENS





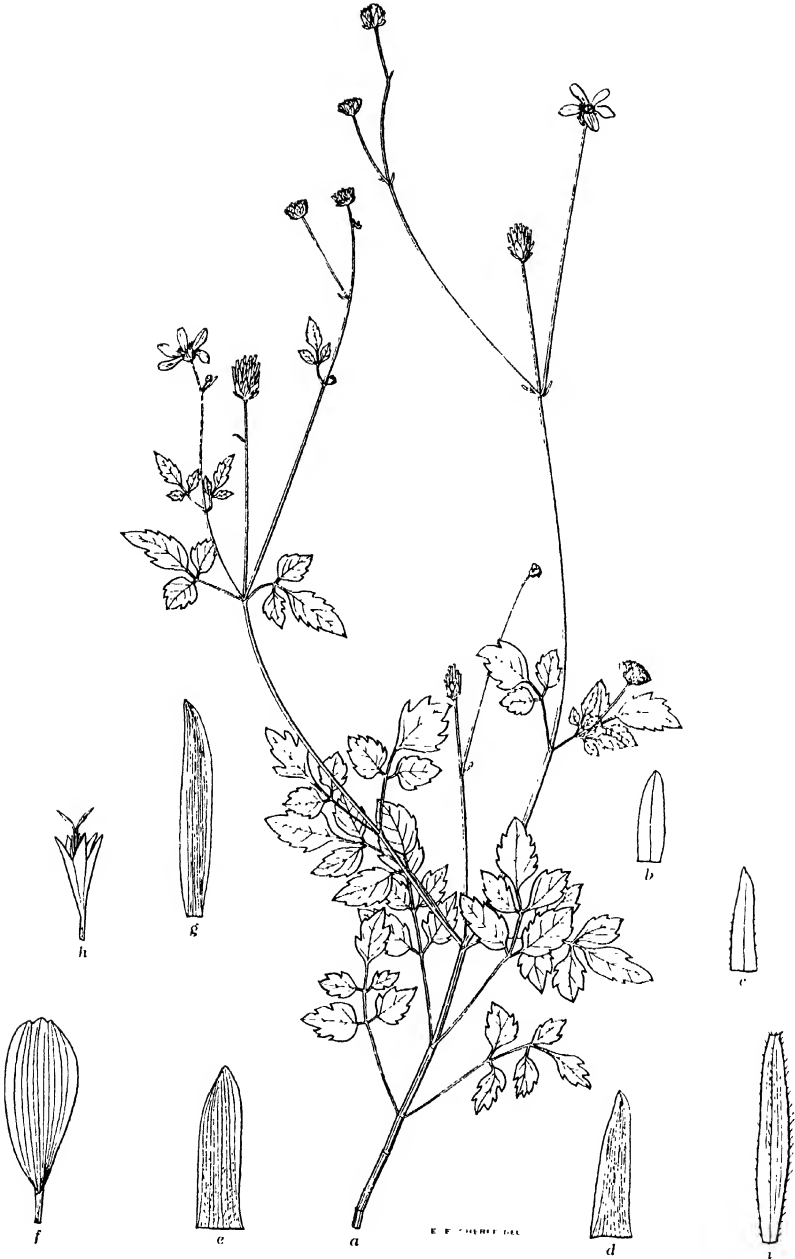
SHERFF on BIDENS





SHERFF on BIDENS

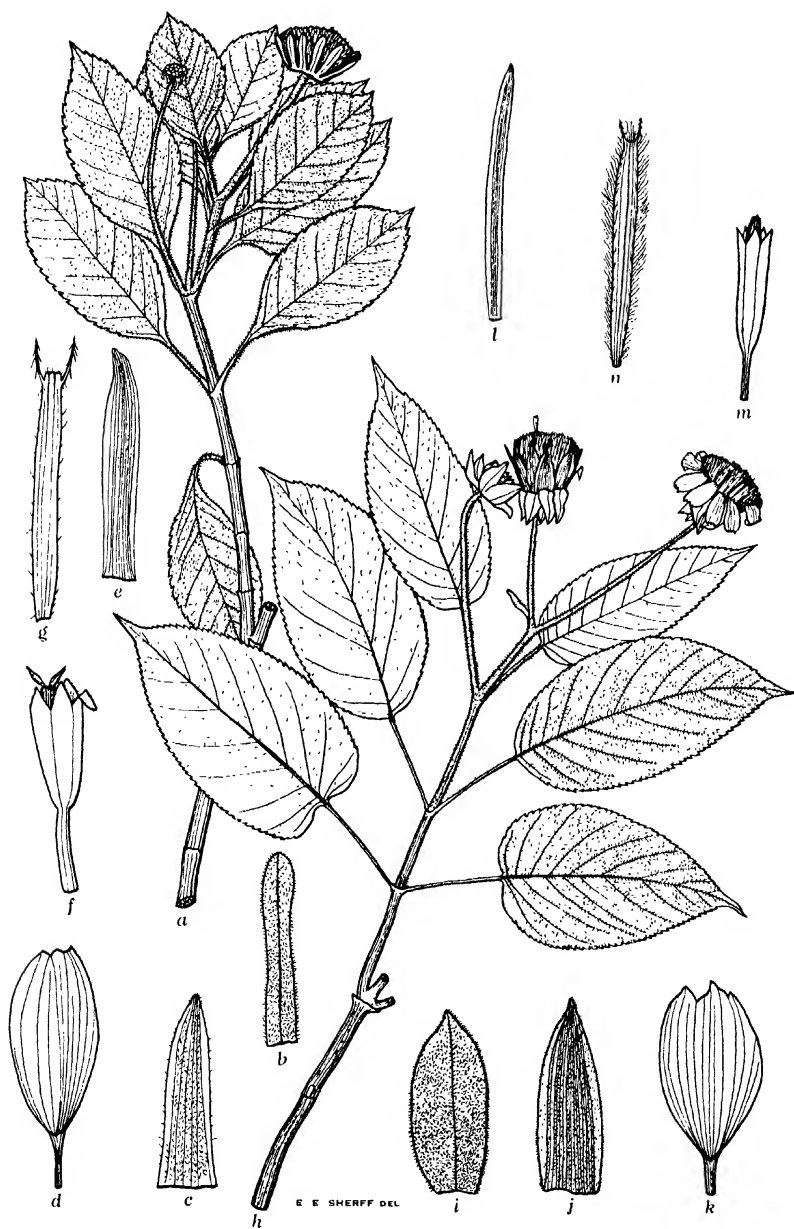




SHREFF on BIDENS









## EXPLANATION OF PLATES I-V

## PLATE I

*Bidens polycephala*: *a*, flowering and fruiting branch,  $\times 0.71$ ; *b*, exterior involucre bract,  $\times 7.1$ ; *c*, interior involucre bract,  $\times 7.1$ ; *d*, ligule,  $\times 7.1$ ; *e*, palea,  $\times 7.1$ ; *f*, disc floret,  $\times 7.1$ ; *g*, *h*, achenes,  $\times 7.1$ ; all from *Ch. Henry*, Mt. Taie Kaoa, Isl. Nukahiva, in 1916, Hb. Field Mus.; *B. ahnnei*: *i*, flowering and fruiting branch,  $\times 0.71$ ; *j*, exterior involucre bract,  $\times 7.1$ ; *k*, interior involucre bract,  $\times 7.1$ ; *l*, ligule,  $\times 7.1$ ; *m*, palea,  $\times 7.1$ ; *n*, disc floret,  $\times 7.1$ ; *o*, *p*, achenes,  $\times 7.1$ ; all from *Ch. Henry*, mountain near Hakau, Isl. Nukahiva, in 1916, 1st type sheet of *B. ahnnei*, Hb. Field Mus.

## PLATE II

*Bidens henryi*: *a*, flowering and fruiting branch,  $\times 0.76$ ; *b*, exterior involucre bract,  $\times 4.5$ ; *c*, interior involucre bract,  $\times 4.5$ ; *d*, ligule,  $\times 3$ ; *e*, palea,  $\times 4.5$ ; *f*, disc floret,  $\times 4.5$ ; *g*, *h*, *i*, achenes,  $\times 4.5$ ; all from *Ch. Henry*, Isl. Hiva-Oa, December, 1917, type in Hb. Field Mus.; *B. pulchella*: *j*, entire plant,  $\times 0.76$ ; *k*, exterior involucre bract,  $\times 6$ ; *l*, interior involucre bract,  $\times 6$ ; *m*, ligule,  $\times 6$ ; *n*, palea,  $\times 6$ ; *o*, disc floret,  $\times 6$ ; *p*, achene,  $\times 6$ ; all from *Adelbert von Chamisso*, Isl. Oahu, in 1816, type of *B. pulchella*, Hb. Berl.

## PLATE III

*Bidens asymmetrica*: *a*, flowering and fruiting branch,  $\times 0.66$ ; *b*, a tripartite leaf,  $\times 0.66$ ; *c*, exterior involucre bract,  $\times 4$ ; *d*, interior involucre bract,  $\times 4$ ; *e*, ligule,  $\times 4$ ; *f*, palea,  $\times 4$ ; *g*, disc floret,  $\times 4$ ; *h*, a 1-awned and *i*, a 2-awned achene,  $\times 4$ ; all from *Abbé Faurie* 960, cotypes in Hb. Brit. Mus. and Hb. Deless.

## PLATE IV

*Bidens graciloides*: *a*, flowering and fruiting branch,  $\times 0.69$ ; *b*, *c*, exterior involucre bracts,  $\times 5.5$ ; *d*, *e*, interior involucre bracts,  $\times 5.5$ ; *f*, ligule,  $\times 5.5$ ; *g*, palea,  $\times 5.5$ ; *h*, disc floret,  $\times 5.5$ ; *i*, achene,  $\times 5.5$ ; all from *C. N. Forbes* 2435 O, type of *B. graciloides*, Hb. Bishop Mus.

## PLATE V

*Bidens lantanoides*: *a*, small branch,  $\times 0.84$ ; *b*, exterior involucre bract,  $\times 4.2$ ; *c*, interior involucre bract,  $\times 4.2$ ; *d*, ligule,  $\times 4.2$ ; *e*, palea  $\times 4.2$ ; *f*, disc floret,  $\times 6.7$ ; *g*, achene,  $\times 4.2$ ; all from *U.S. Explor. Exped. (Capt. Wilkes)*, Isl. Eimeo, *a* from type in U.S. Nat. Hb.; *b-g*, from cotype in Hb. Gray; *B. cordifolia*: *h*, small branch,  $\times 0.84$ ; *i*, exterior involucre bract,  $\times 4.2$ ; *j*, interior involucre bract,  $\times 4.2$ ; *k*, ligule,  $\times 4.2$ ; *l*, palea,  $\times 4.2$ ; *m*, disc floret,  $\times 6.7$ ; *n*, achene,  $\times 4.2$ ; all from *Barclay* 3213, Isl. Nukahiva, January, 1840, Hb. Brit. Mus.

# A MORPHOLOGICAL COMPARISON OF LEAFLETS OF A HYBRID CYCAD AND THE TWO PARENTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 376

SOPHIA PAPADOPOULOS

(WITH TWENTY-ONE FIGURES)

## Introduction

A generic cross between *Ceratozamia mexicana* and *Zamia monticola* was described by CHAMBERLAIN (3) more than a year ago. About fifty of the hybrids are growing vigorously, and Professor CHAMBERLAIN suggested that I make a detailed study of the two parents and the hybrid, to determine what features of the parents can be recognized in the  $F_1$  generation. I am indebted to him for material and criticism, and wish also to thank Professor LAND for his interest in the work.

The two parents, *Z. monticola* and *C. mexicana*, have been growing luxuriantly for many years in the University of Chicago greenhouse, where they have been under constant observation. *Z. monticola*, the new species described by CHAMBERLAIN (2), was grown from a seed looking like a *Ceratozamia* seed and picked up in a *Ceratozamia* region near Jalapa in Mexico; but upon germination it proved to be a *Zamia*. This is a male plant and produces cones regularly. No female cones of this species are known. Seeds of *C. mexicana* were also brought from the same region, and plants grown from them have proved very hardy and have produced cones.

In April and May, 1924, two cones of *C. mexicana* plants were pollinated with the pollen of two cones from the plant of *Z. monticola*. In November, 1924, these two cones produced a great number of seeds, nearly one hundred, most of which were planted within a period of a few days. These seedlings were repotted in July, 1925, and have since been growing vigorously. The topography and contour of the leaflets of these hybrids have been described by CHAMBERLAIN (3).

Comparatively little work has been done in the anatomy of the leaflets of cycads. The most recent work is that of LAMB (9), who described some features of the anatomy of the leaflets in an attempt to determine whether genera and species of cycads might be determined by the character of the leaflets. In her paper she describes *Z. monticola* as "Z. II," which was then an unidentified species. She also describes the leaflets of *C. mexicana*. KRAUS (8) gives a rather detailed description of the histological characteristics of *C. mexicana*. POOLE (11) in his comparative study lays stress on petioles rather than upon the leaflets.

### Methods

For the anatomical study of the leaflets, freehand sections were made through the tip, base, and middle. Younger and older leaflets of *C. mexicana* were sectioned for a more exact study, since the younger ones were more succulent. Only one group of *Z. monticola* leaflets was sectioned, since they were of approximately the same age. In the hybrid, leaflets of the first and second leaves were sectioned separately. In cutting, the tips of several leaflets were put together and bound with a string. These were then placed in a sliding microtome and cut with a razor. With a sharp razor some fairly thin sections can be obtained even though the leaflets are coriaceous.

After being cut, the sections were placed in formalin-alcohol-acetic fixing agent for 24 hours. The proportions used were 10 cc. of commercial formalin, 5 cc. of glacial acetic, and 85 cc. of 50 per cent alcohol. At the end of 24 hours the sections were washed in 50 per cent alcohol for one hour, the alcohol being changed twice.

Two methods were used in staining, safranin with light green and aniline blue with phloxine. The 50 per cent alcoholic safranin was used and specimens were stained for 24 hours. They were then washed, one minute each, in 50, 70, and 85 per cent alcohol respectively. The light green was in 90 per cent alcohol and was very easily absorbed by the leaflets, so that four or five seconds was sufficient for thorough staining. They were then washed in 95 per cent alcohol for one minute, in absolute alcohol for one minute (changing once), in clove oil for one minute or more, in xylol for two or three minutes; they were then mounted in balsam.

Those stained with Magdala red and aniline blue were first

placed in 90 per cent alcoholic solution of phloxine for 24 hours; then dipped in 95 per cent alcohol and stained three or four seconds in aniline blue; then treated a few seconds in 96 per cent alcohol with HCl (a watch-glass full of alcohol with two or three drops of HCl); after which they were washed in 95 per cent alcohol, dehydrated, cleared, and mounted in balsam. Since the acid drew the phloxine very rapidly, some sections were left in that stain for 48 hours.

### Description

#### CERATZAMIA MEXICANA

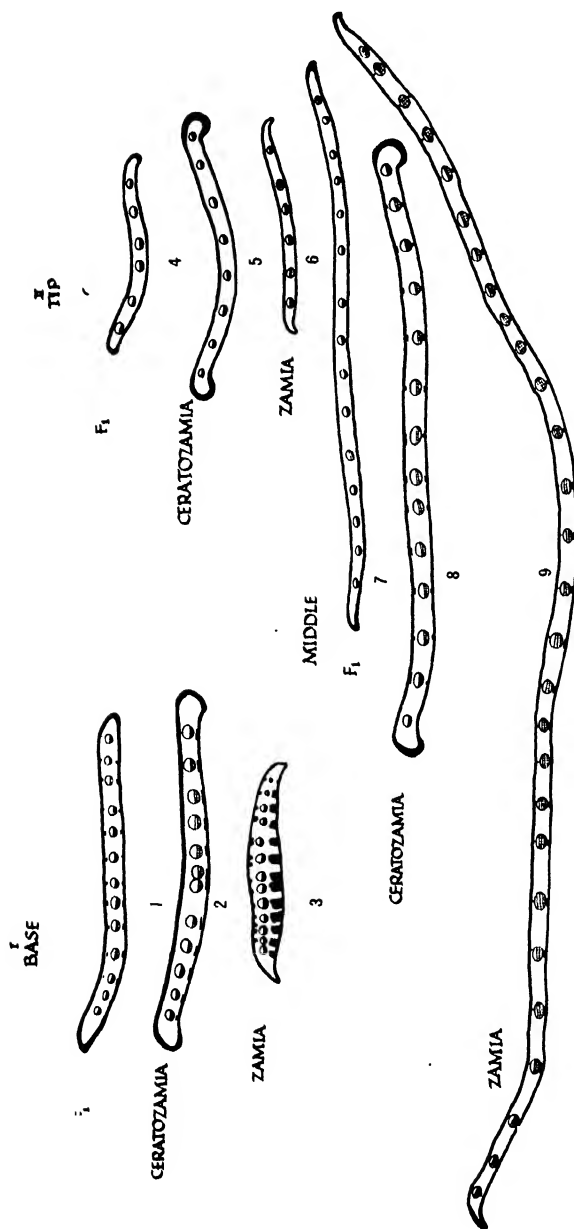
The leaflet of *C. mexicana* is entire, and is described in the manuals as "integerrima." It is narrow, tapering to a rather sharp point at the tip; toward the base there is a gradual decrease in the width of the pinnule to about one-half the width. The surface of the leaflet is very highly cutinized and the edges are revolute. A transverse section through the middle portion of the younger leaflets of *C. mexicana* shows the following characteristics.

The cuticle, which covers the entire surface of the leaf, is thicker on the upper surface of the leaflet than on the lower. The epidermal cells are rather elongated, and pointed with evenly thickened walls. KRAUS describes them as being shaped like prosenchyma cells. The degree of thickening varies in the different cells, and in some of them the lumen entirely disappears, especially in those cells immediately below the bundle in the lower epidermis and those at the revolute edge. The size of the epidermal cells varies; they are more uniform on the upper surface, while on the lower surface they are larger between the bundles and more uniform immediately below the bundles. Both the upper and lower epidermis are interspersed with thin-walled oval cells resembling glands, occurring singly or in pairs. These cells are filled with granular cytoplasm and have large nuclei.

Numerous stomata of a complex type are found in the lower epidermis. They are arranged irregularly through the epidermis, occasionally occurring in groups of two and three, then at times occurring at long intervals.

The number of stomata in a transverse section of the different regions in the leaflets is shown in table I.

The stoma in longitudinal section is a difficult structure to de-



FIGS. 1-9.—Contour of transverse sections through leaflets, showing topography of bundles and regions of sclerenchymatous tissues (in black): 1, transverse section through base of pinnules of *F<sub>1</sub>*; 2, transverse section through base of pinnules of *Ceratizamia*; 3, transverse section through base of *Zamia* leaflet; 4, transverse section through tip of *F<sub>1</sub>* pinnules; 5, transverse section through tip of *Ceratizamia* pinnules; 6, transverse section through tip of *Zamia* pinnules; 7, transverse section through middle of *F<sub>1</sub>* pinnules; 8, transverse section through middle of *Ceratizamia* pinnules; 9, transverse section through middle of pinnules of *Zamia*;  $\times 7$ .



scribe. There are two distinct guard cells immediately below the epidermis. These are very much thickened except at the "hinges," where the wall is quite thin. Where the two guard cells meet, there is a slight curvature and indentation in the beaklike tip. The stomatal opening is rather broad, and at the base near the sides are thickenings. KRAUS describes the stoma in surface view as being surrounded by isodiametric cells, which give the opening an oval shape parallel with the leaf. Above these guard cells are three others, arranged diagonally, which may be considered as auxiliary guard cells.

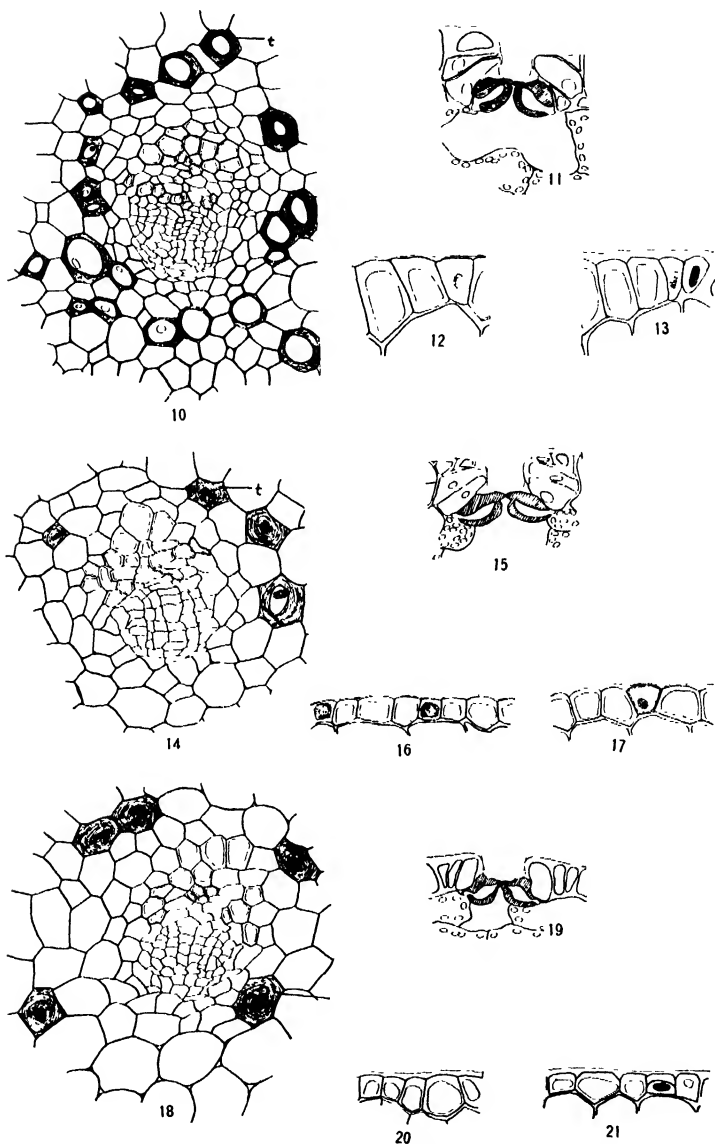
TABLE I

REGION	AVERAGE	MAXIMUM	MINIMUM
Younger leaflets			
Middle.....	16	29	10
Tip .....	10	18	4
Base.....	14	22	9
Older leaflets			
Middle.....	27	33	21
Tip.....	11	16	7
Base.....	15	23	10

The two immediately above are triangular with thin walls, whereas the ones at the surface have thickenings and very much resemble guard cells.

Thickened cells occur regularly immediately below the bundles on the lower epidermis, except in those bundles nearest the revolute edge, where these cells may be present in one or two cells, or may be lacking entirely. On the upper surface these thickened cells form in small patches one layer in thickness in the older leaves; in the younger leaves they occur in single cells or in groups of four or five cells. These cells are similar in shape to the epidermal cells, except perhaps that they are rather rounded. The thickness of the walls is even, leaving the lumen very distinct.

The mesophyll consists of two distinct regions, the palisade and the spongy tissue. The palisade is a single layer of rather elongated, compact chlorophyll-bearing cells with the long axis perpendicular



FIGS. 10-21.—Details of bundles, stomata, upper and lower epidermis (*t*, lignified tracheids): 10, vascular bundle of *Ceratozamia*; 11, stoma of *Ceratozamia*; 12, lower epidermis of *Ceratozamia*; 13, upper epidermis of *Ceratozamia*; 14, vascular bundle of *F1*; 15, stoma of *F1*; 16, upper epidermis of *F1*; 17, lower epidermis of *F1*; 18, vascular bundle of *Zamia*; 19, stoma of *Zamia*; 20, lower epidermis of *Zamia*; 21, upper epidermis of *Zamia*;  $\times 350$ .

to the upper epidermis. Below the palisade are two rows of elongated cells of about the same size, which are parallel to the surface. The spongy tissue consists of cells elongated to about three or four times their width, arranged regularly, and parallel to the surface. Some of these cells are yoke-shaped, and where they come together leave oval intercellular spaces. The yoke-shaped cells are more frequent in the older leaves than in the younger ones. KRAUS describes the region below the palisade as palisade parenchyma, and mentions that in the middle of the palisade parenchyma lie the vessels and the bast cells, held together by the cross parenchyma. In this spongy tissue are present sclerenchyma cells with thickened angular walls. These cells are arranged parallel to the palisade region, and occasionally occur in the lower mesophyll. The number of these cells between the bundles varies; in the older leaflets the average near the tip is four, while the maximum number is ten and the minimum two. Toward the base the average is six, while the maximum and minimum numbers are fourteen and four respectively. Through the middle the average is six, while the maximum is eleven and the minimum four. The number of these lignified cells between the bundles is approximately the same in the older and younger leaflets. At the revolute edge they are evenly distributed in the parenchyma.

These thick-walled "bast" cells, found between the bundles and also surrounding them, were found to stain differently in some of the sections. This variation in the staining proved to be due to poor fixation, but suggested a test to determine the nature of this thickening. Several tests for suberin, cutin, and lignin were made.

A 5 per cent aqueous solution of phloroglucin, applied simultaneously with hydrochloric acid, gave all these thick-walled cells a reddish violet color. This test was made with a shaving of wood for comparison, and the color was so identical that it immediately suggested that these walls were lignified. In order to verify this, the solubility and oxidation tests were also tried. The thickenings completely disappeared upon the application of 50 per cent chromic acid; another indication of lignin. The oxidation test with nitric acid and hydrogen peroxide also showed that the membrane had completely disappeared.

Only one test was made for suberin, the iodine and sulphuric acid, but since it showed no reaction at all, the lignin tests were applied and showed quite distinctly that these thick-walled cells in the mesophyll are lignified like the thickened epidermal and hypodermal cells.

Between these bundles, as well as in the spongy tissue, are mucilage canals varying in number from four to none at all. Calcium oxalate crystals are found in the long spongy cells, and occasionally

TABLE II

REGION	AVERAGE	MAXIMUM	MINIMUM
Younger leaflets			
Middle.....	11	13	9
Tip.....	8	10	6
Base.....	11	14	6
Older leaflets			
Middle.....	11	14	9
Tip.....	5	9	4
Base.....	10	13	7

in the palisade cells. As many as four have been observed in one of the long cells. Stone cells for strengthening are also found in the mesophyll.

The vascular bundles are arranged at even intervals in the spongy parenchyma almost through the center of the leaflet, just a little nearer the lower surface. About eighty sections each, through the tip, middle, and base of the younger and older leaflets were examined. The number of bundles in the middle, tip, and base of the leaflets is shown in table II. There seems to be little variation in the two groups except in the tips of the younger leaflets, where the average is eight.

Each bundle is surrounded first by thickly lignified cells, which are more numerous at the base, and by two or three rows of parenchyma cells. The sclerenchymatous cells have angular walls and laminated thickenings. Some of the walls are so thickened that the lumen entirely disappears. KRAUS describes the bundles as being

surrounded by bast and sieve parenchyma cells, but the tests show quite clearly that these walls are lignified. The bundle is collateral, with the phloem toward the lower surface, arranged in even ladder-like cells with the protophloem at the lower end. The bundle of the pinnules is exarch toward the tip, whereas at the base, near the point of attachment, it is mesarch. A slight variation may occur, since occasionally a mesarch bundle occurs beyond the middle. HILL and DEFRAINE found that in general the bundles of the cotyledons are mesarch, and then become exarch. DORETY, in her study of *Microcycas calocoma*, describes the wood as endarch as far out as the sheathing base of the cotyledon, where it becomes mesarch, while the greater number of strands are mesarch toward the top of the blade and then exarch. This condition seems to hold true also in these pinnules. There is a definite protoxylem point in the center, with the metaxylem above, but in many of the bundles the parenchyma comes up through the xylem on both sides, cutting it into three parts and giving it the appearance of having three protoxylem points.

#### ZAMIA MONTICOLA

The leaflets of *Z. monticola* are sometimes entire and sometimes serrate near the tip, but are more generally entire in this species. They are quite broad through the center and taper down at the base to about one-fifth their width; at the top there is an abrupt tapering to a sharp point, which gives the leaflet a slightly curved tip. The surface of the leaflets is highly cutinized, especially on the upper epidermis. The epidermal cells are quite uneven in size and thickness, and are larger on the lower epidermis, with thicker walls at the top, giving the lumen a more rounded surface toward the top. The walls of the epidermal cells are rarely thickened to the extent of having the lumen disappear, even at the edge of the leaflet. The edge of these leaflets tapers out rather than being rounded as in *Ceratozamia*.

In the upper epidermis, and only occasionally in the lower, are found some peculiar cells with a very thick upper wall. They occur frequently on the upper surface and are found singly, in pairs, or in groups. These thickenings stain with phloxine, taking about the same color as the thickenings of the guard cells. None have been

observed to form stomata, but where they are arranged in pairs they suggest incompletely developed stomata. The nucleus and protoplasm of these cells have also taken a little of the red stain, showing that they were probably becoming disorganized.

Numerous sunken stomata are scattered in the epidermis with the guard cells immediately below the epidermis. There is considerable variation in the number of stomata in the tip, base, and middle of the leaflets. Table III gives the average number.

Thickened differentiated hypodermal cells occur above and below the bundles, developing toward the bundles rather than being arranged parallel to the epidermis, as in *Ceratozamia*. Only two or three rows of these thickened cells occur at the edge of the leaflet.

TABLE III  
NUMBER OF STOMATA IN *Z. MONTICOLA*

REGION	AVERAGE	MAXIMUM	MINIMUM
Middle . . . . .	89	112	61
Tip . . . . .	12	22	8
Base . . . . .	11	25	6

The palisade layer is not as highly differentiated as is that of *Ceratozamia*. The cells are not distinctly elongated and usually appear rounded.

The spongy mesophyll is very distinct, and also consists of yoke-shaped cells elongated to five or six times their width. When these cells come together they leave an opening varying from a small oval to larger, irregularly shaped spaces. The amount of chlorophyll in these cells varies, but is much greater in those near the palisade than in the layers below.

No thick-walled lignified cells were found in the mesophyll, but stone cells are more numerous. Calcium oxalate crystals are also found through the spongy mesophyll, but are not as numerous as in *Ceratozamia*. Mucilage ducts have not been found to occur in these leaflets.

The bundles of *Z. monticola* are not as large as those of *Ceratozamia*, and are not as strong. They are not surrounded by sclerenchyma cells, although a few are present and are more numerous at

the lower end. The bundles near the base are held to both surfaces by numerous lignified cells, which become fewer and fewer toward the top, leaving the bundles surrounded by two or three rows of parenchyma cells. The bundle is mesarch toward the base of the leaflets, and exarch toward the tip; but mesarch bundles have been found occasionally beyond the middle of the leaflet. There is very little xylem in these bundles, only two or three protoxylem cells and from five to about ten metaxylem cells. The phloem cells are quite even, with the protophloem at the base, and they do not have the crushed appearance of the phloem cells of *Ceratozamia*.

The bundles are evenly distributed throughout the middle, base, and tip, as shown in table IV.

TABLE IV  
NUMBER OF BUNDLES IN *Z. MONTICOLA*

REGION	AVERAGE	MAXIMUM	MINIMUM
Middle.....	23	30	20
Tip.....	8	11	6
Base.....	14	18	9

#### FIRST LEAVES OF $F_1$ GENERATION

The first leaflets are entire, rather short and wide, resembling *Zamia* in contour, with the somewhat abrupt tapering at the tip. At the base there is a gradual decrease in width to about one-fifth the maximum width at the middle.

The surface of the leaflets is highly cutinized, the cutin being thicker at the tip than at the base. The upper epidermis consists of rather large, thickened cells of unequal size and thickness. The thickening is greater on the upper and lateral surfaces, but at the base the cell comes to a point. Thickened hypodermal cells immediately below the bundle, parallel to the surface and in groups developing toward the bundle, occur only toward the base of the leaflets. These cells also occur at the revolute edge, varying in number from two to four rows. The edge of the first leaflets varies. Some sections show gradual tapering, with a rounded edge; while in others the narrowing continues, giving it a slightly rounded edge; and some are rounded without any narrowing.

Between the epidermal cells, at regular intervals of four or five

cells, are some very conspicuous cells which have thin walls, very large nuclei, and are full of granular cytoplasm. The walls of the lower epidermis are very much larger, and vary in size and thickness. The thickening here also is greater at the upper and on lateral surfaces, with the pointed thin wall at the base. The lumen of these cells is large and distinct. The thin-walled cells with the large

TABLE V  
NUMBER OF STOMATA IN F<sub>1</sub> GENERATION

REGION	AVERAGE	MAXIMUM	MINIMUM
Middle.....	24	26	15
Tip.....	4	8	2
Base.....	6	11	3

nuclei in the upper epidermis are also present in the lower epidermis, but are not quite as numerous.

The stomata are very large and distinct, with the guard cells immediately below the epidermis, and resemble those of both *Ceratosamia* and *Zamia*. Table V shows the distribution of the stomata in the different regions of the pinnules of the F<sub>1</sub> generation.

TABLE VI  
LEAFLETS OF F<sub>1</sub> GENERATION

REGION	AVERAGE	MAXIMUM	MINIMUM
Middle.....	14	16	13
Tip.....	4	6	3
Base.....	10	15	7

The bundles are rather small and are arranged at regular intervals in the spongy mesophyll nearer the lower surface. They are surrounded by large parenchyma cells in which lignified cells are sometimes present, varying in number from six to none at all. At the base these sclerenchyma cells surround the bundle completely. Table VI shows that the bundles are more numerous through the center of the leaflet, while at the tip and the base the numbers are approximately the same. The bundles near the base are mesarch, while toward the tip they are exarch. The xylem in these bundles is not very highly lignified except in those near the base, which are



larger and have a greater proportion of xylem and phloem. The phloem cells are arranged generally in ladder-like form, but some appear quite crushed and irregular.

#### SECOND LEAVES OF $F_1$ GENERATION

The second leaflets are longer and broader than the first, and taper to a sharper point. The mesophyll shows very distinct differentiation of palisade and spongy mesophyll. The palisade consists of large, wide, and elongated cells full of chlorophyll, and with large, distinct nuclei. The large, yoke-shaped cells immediately below are also rich in chlorophyll. The length of these cells is six to seven times their width, and they are so rich in cytoplasm that they have an inflated appearance. There are larger intercellular spaces through the spongy mesophyll, as these cells are more deeply yoked.

Large mucilage canals and also calcium oxalate crystals appear in this spongy mesophyll. In the palisade tissue crystals are frequently found in these leaflets. Stone cells and thick-walled lignified cells are also found occasionally between the bundles.

The contour of most of the leaflets is entire, but two of those sectioned have one notch near the tip, which suggests serration.

The histological characteristics of these leaflets is the same as in the leaflets of the first leaf, except that the spongy mesophyll in most of the sections observed is more compactly arranged, in almost parallel rows.

#### Comparison

The comparison of *Zamia monticola* and *Ceratozamia mexicana* with the  $F_1$  generation cannot be entirely satisfactory, because it is necessary to compare the seedlings of the  $F_1$  generation with the adult parents. Seedlings of the parents are not available.

Upon germination, the seeds of the  $F_1$  generation had two cotyledons, a *Zamia* characteristic; while numerous *Ceratozamia* seeds germinated here twenty years ago showed only one cotyledon. Seeds germinating as they fall naturally on the ground, probably always have only one cotyledon. DORETY (6) produced two cotyledons by germinating on a clinostat, and a few cases have been reported where the two cotyledons developed probably because the seeds were planted in an unnatural position.

A comparison of the leaflets of the first and second leaves shows the leaflets of the first leaf entire and somewhat narrow, although having the contour of the *Zamia* leaflet, with its abrupt tapering to a point. On the other hand, the leaflets of the second leaf occasionally show a suggestion of serration. Leaflets after the second ones also show a slight curving, and they sometimes show single notches.

Transverse sections of both the first and second leaflets show the revolute edge with the characteristics of both parents. Some leaflets are especially interesting, showing a narrowing toward the edge of the leaf, a *Zamia* characteristic, and just at the tip becoming wider and more rounded with three to five rows of lignified cells, which is a *Ceratozamia* characteristic. The same leaflet then shows the characteristic of both parents.

On the whole the epidermal cells show greater thickening at the top and sides, with the thin, rather pointed lower wall, this being distinctly characteristic of *Zamia*.

The epidermis of both the first and second leaflets is interspersed, at almost equal distances, with these thin-walled cells, like the glands found in *Ceratozamia*. These cells are rich in granular protoplasm and have large nuclei. With the aniline blue and phloxine stain the epidermal cells are red, with these cells blue, and they appear very distinctly, while in *Ceratozamia* the blue is not so distinct, probably on account of the disorganization of the cell contents.

The cells of the seedlings proved to be larger and with more protoplasm, while the chlorophyllose cells were filled with large chloroplasts. This condition might have been interpreted as hybrid vigor had we the seedlings of the two parents for comparison, but as it is we can come to no conclusion, since the seedlings of the parents may show the same vigor.

Thickened differentiated hypodermal cells were found only at the base of the leaflets, while toward the top they are replaced by chlorophyll-bearing cells. These thickened hypodermal cells are arranged in such a way as to resemble both parents. On the same leaflet they are observed to be arranged in a single row, parallel to the surface, immediately below the bundles just as in the *Ceratozamia*; while at the top they are found in groups, developing toward

the bundle as in *Zamia*. Others have been observed to be very much as in *Zamia*, in groups, coming toward the bundle.

The bundles near the end are distinctly surrounded by lignified cells arranged in the same position as those of *Ceratozamia*, while toward the top there is a gradual decrease in the number of lignified cells replaced by large parenchyma cells, which is a *Zamia* character. The bundles also are smaller and weaker as a whole, toward the top, with less phloem and with the xylem not very highly lignified.

Mucilage canals as numerous as those in *Ceratozamia* are found between the bundles, while none have been observed in the *Zamia* leaflets. Thick-walled lignified cells varying in number from four to ten, which occurred regularly between the bundles of *Ceratozamia*, appear occasionally in the hybrid leaflets, not exceeding three in number.

Stone cells are found in both parents and in the hybrid, but appear more numerous in *Zamia*, probably for strengthening the large, broad leaves, since the bundles are quite weak.

The stomata of the hybrid show a very interesting development. Those of *Zamia* are slightly sunken, the guard cells having very thick walls, and the pointed ends, which come together, curving upward with two accessory cells. In *Ceratozamia* the stoma is more sunken, with three accessory cells, while the lumen of the guard cells is more distinct. The stomata of the hybrid are somewhat sunken, with two accessory cells, while the guard cells are not as greatly thickened, with a very distinct lumen. The general contour of the guard cells resembles stomata of both parents. Where they come together there are the slightly pointed ends curving upward as in *Zamia*, while immediately below are the two little notches found in *Ceratozamia*. There is also that slight thickening in the lower corners of the stomatal opening present in both parents.

More definite conclusions could be drawn if seedlings of both parents had been available; it is quite clear that the  $F_1$  generation has inherited, rather equally, characteristics of both parents.

### Summary

A morphological study of the leaflets of a hybrid cycad obtained from a cross between *Ceratozamia mexicana* and a new species,

*Zamia monticola*, shows that some of the structures distinctly resemble either one parent or the other, and that other structures show characteristics of both parents. The stomata illustrate the latter point clearly, as they combine distinctly the peculiarity found in the contour of the guard cells in both parents.

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## SOME MISUNDERSTOOD PIPERACEAE<sup>1</sup>

LISLE W. MCKOWN

(WITH THREE FIGURES)

### I. PIPER CHINANTLENSE MART. AND GAL.

Among the plants collected in Mexico by GALEOTTI was a *Piper* (Gal. 6020) found near the town of Chinantla, on the east slope of the eastern Cordilleras in the Valley of Oaxaca, at an elevation of 3000 feet. It was named and described thus by MARTENS (18):

"*Piper chinantlense*. Nobis. (Coll. H. Gal. no. 6020) — Glaberrimum; caule fruticoso, foliis alternis venoso-nervosis coriaceis ovato-lanceolatis acuminatis nitidis basi inaequaliter subcordatis petiolo canaliculato alato caulem amplexantibus, spicis axillaribus solitariis breviter pedunculatis petiolo sesquialongioribus. — Folia 5-6 pollices longa, 3-4 pollices lata. — Affine *Piperi jalapensi* nobis, sed folia subtus glabra."

"On trouve cette espèce dans les belles forêts de la Chinantla, région située sur le versant oriental de la cordillère orientale d'Oaxaca, à 3000 pieds."

The same year and the following, 1843-1844, MIQUEL published his *Systema Piperacearum*. The work of MARTENS and GALEOTTI had evidently been brought to his attention after his book was in the hands of the printer, for he points out in his "corrigenda et addenda" that MARTENS and GALEOTTI'S *Piper chinantlense* is probably synonymous with his own *Artanthe caladiifolia*, his genus *Artanthe* now being classed as a subgenus under *Piper*. He points out (20), however, that in his species the spike is only very slightly axillary: "Page 387. N. 9. adde syn.: *Piper chinantlense* Mart. et Gal. 1. c. p. 387, sed amenta minime axillaria, ut dicunt."

In 1869, twenty-five years later, C. DE CANDOLLE (4) included *Piper chinantlense* among a number of species which he considered doubtful, or which he thought should be excluded. He evidently

<sup>1</sup> The substance of a thesis in candidacy for the master's degree at the University of Illinois, 1926.

took his description from that of MARTENS and GALEOTTI, for reference is made to their original description, and he does not state that he had seen a specimen. HEMSLEY (14) in 1882-86 merely mentions *P. chinantlense*, and refers to MARTENS' description and to DECANDOLLE'S reference in the *Prodromus*. *P. chinantlense* is not mentioned even in the index in DECANDOLLE'S analytical key to the Piperaceae (6), posthumously published in 1923. The peculiar distinction in the description given by MARTENS is the axillary spike, rather than the spike opposite the leaf, the latter being characteristic of all other true Mexican species of *Piper*. It has already been stated that MIQUEL noted this peculiarity. The species has not been recognized subsequently.

BOURGEAU in 1865 found a *Piper* (Bour. "1898") in the valley of Cordova, above Vera Cruz, northeast of Chinantla, but in about the same botanical region. C. DECANDOLLE named this *Piper cordovanum* and described it thus (5):

"*Piper cordovanum*.—Foliis petiolatis oblongis apice acuminatis acumine acuto basi inaequali obtusis obscure viridibus, membranaceis opacis pellucido-punctulatis utrinque glabris, nervis subtus prominulis centrali tota longitudine nervos utrinque 8-10 suboppositos subadscendentes mittente quorum supremi ad apicem usque ducti petiolo glabro ad limbi latius minus usque vaginante. Amento quam folium multoties breviote mucronato, pedunculo glabro quam petiolus multum breviore. Bractea cucullata glabra, staminib. 4, antheris obtusis stigmatib. 3, linearibus. In valle Cordova (Bourgeau! 1898 in herb. DC.). Ramuli glabri subtetragoni. Limbi 0,165 longi, 0,07 lati. Petioli 0,035 longi. (v. s.)"

ROVIROSA in 1889 collected a similar *Piper* near San Sebastian, Tabasco, which C. DECANDOLLE named *P. rovirosae* and described thus (7):

"*Piper rovirosae* C. DC., n. sp.—Omnino glabrum; foliis modice petiolatis, limbo subovato-oblongo, basi subaequilatera cordato apice breviter acuminato acumine obtusiusculo et breviter mucronulato; nervo centrali nervos arcuatos adscendentes et sat distantes utrinque 5 emittente quorum supremus paullo supra  $\frac{2}{3}$  longitudinis centralis solutus, petiolo usque ad limbum vaginante; pedunculo quam petiolus breviore, spica quam limbus pluries breviore apice

mucronata, bractee vertice anguste inflexo, antheris rotundatis quam filamenta paullo longioribus, bacca libera subtetragona, stigmatibus linearibus. . . . Limbi in sicco rigiduli minute pellucido-punctulati, usque ad 14,5 cm. longi 6,5 lati. Petioli 2 cm., pedunculi 7 mm. longi. Spica matura 3 cm. longa et 2 mm. crassa, ejus mucro fere 2 mm. longus; stamina 4 basi ima baccae adnata, bacca in sicco nigra stigmata 3 sessila. Sebastian in umbrosis, Aprili, vern. Cordoncillo (Rovirosa n. 473, h. Donn. Sm.)."

BUCHINGER at some unascertained time before 1869 collected a *Piper* near Oaxaca, southeast of Chinantla, which DECANDOLLE in the *Prodromus* named *P. rohrii crassum*. He also states that *P. rohrii* equals *Steffensia ampla* Kunth and *Artanthes ampla* Miquel, a native of Brazil. C. DECANDOLLE's description of *P. rohrii crassum* is as follows (4):

"*Piper rohrii*.—Foliis petiolatis lato-ellipticis apice breviter acuminatis, acumine acuto, basi subaequali subattenuatis utrinque glabris opacis rigido-membranaceis, nervo centrali subtus prominente utrinque tota longitudine nervos circ. 15 suboppositos subtus prominulos subpatulos mittente, petiolo glabro paullo ultra limbi basin vaginante, alis linearibus, pedunculo glabro quam petiolus brevior, amento maturo quam folium brevior, bractee cucullatae basi ciliatae vertice triangulari inflexo peltam ciliolatam simulante, bacca glabra. . . . Ramuli glabri. Limbi 0,21–0,23 longi, 0,11 circ. lati. Petiolo 0,025 longi. Pedunculi 0,012 longi. Amenta mat. 0,09 circ. longa, 0,0035 crassa. (v. s.)"

"β, Crassum, amentis crassioribus, maturis 0,007 crassis, 0,09 longis.—In Oaxaca (Buchinger! in H. Lenormand). (v.s.)"

These four nominal species of *Piper* parallel one another in description except at two places, the leaf size and shape, and the position and size of the spike. The size limits of the first three are practically the same. *Piper rohrii crassum*, however, is distinctly larger. The shape of the leaves may vary slightly within the species, which may account for the variations in the descriptions. It is also possible that the authors had the same general shape in mind, even though the wording of their descriptions varies slightly. The second difference relates to the position and size of the spike. *Piper chinantlense* (Gal. 6020) is the only species of *Piper* reported from Mexico

with axillary spikes. A drawing made by Mademoiselle DURAND for TRELEASE from an original specimen of *P. chinantlense* in the herbarium of the botanical garden at Brussels shows that the spikes really are opposite the leaves, and not axillary, a fact verified on the same material by TRELEASE (fig. 1). *P. rohrii crassum* has larger spikes, but the leaves are also larger, and it is possible that this particular plant (BUCHINGER) grew in a more favorable environment, making it abnormally large, or that it is at least varietally separable from *P. chinantlense*.

The species of *Piper* here mentioned came from the same general region in Mexico, the eastern slope of the eastern Cordilleras, between the parallels of 16° and 20° North Latitude, and at a rather low altitude. *P. chinantlense* may appear to be an exception, but it should be remembered that the Cordilleras are relatively low in this particular region, not exceeding 5000 feet. Chinantla is on the eastern slope of a short spur running south or southwest of the main range of the Cordilleras, and is at an elevation of about 3000 feet; hence it might very properly be said to come within the region described.

Herbarium specimens of these species of *Piper* have been observed by TRELEASE in the following collections:

*Piper chinantlense*.—Near Cordoba, Vera Cruz (Bourgeau 1998 [noted as 1898], in the Candolle Herbarium at Geneva; also 1988

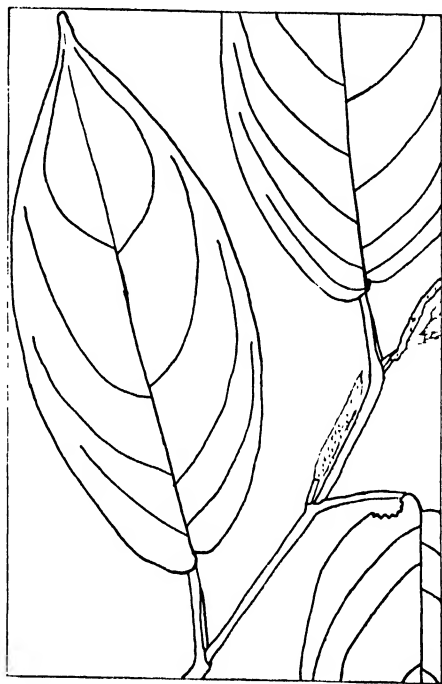


FIG. 1.—Sketch of drawing by Mademoiselle DURAND for Dr. TRELEASE of *Piper chinantlense* (Galeotti 6020), in Univ. Ill. Herb.; from original at Botanical Garden, Brussels.



in U.S. Nat. Herb.; New York Bot. Gard.; the Copenhagen Bot. Gard.; and at the Muséum d'Histoire Naturelle at Paris,—the cotypes): near Orizaba, Vera Cruz (Bourgeau 1353, 1528 at Paris; Botteri and Sumichrast 186 at Paris; Gouin, at Paris): near Mirador, above Jalapa, Vera Cruz (Endlich 1153, at the Bot. Gard. Dahlem, Berlin; Seler at Dalhem): near Coatzacoalcas, Vera Cruz (C. L. Smith 1166, the leaves more broadly elliptical,  $8 \times 16$  cm., rather deeply cordulate, in the U.S. Nat. Herb.; New York Bot. Gard.): near Motzorongo, Vera Cruz (Nelson 157, U.S. Nat. Herb.).

*Piper rohrii crassum*, reported in type description from near Oaxaca, Oaxaca (Buchinger in Herb. Le Normand), is known only from the one collection.

Another *Piper* at present undescribed was collected near Coahuayutla by EMRICH. This specimen is quite similar to the foregoing, except for the very short spikes. Coahuayutla, moreover, is west of the general locality in which the other specimens were found, and in a different botanical region. It is probable, therefore, that it is a different species.

From the facts assembled it seems reasonable to conclude that *Piper rovirosae* of the state of Tabasco is hardly separable from *P. cordovanum* of the state of Vera Cruz, and that this is clearly the same as *P. chinantlense* of the state of Puebla; hence all should be known as *P. chinantlense*, which has priority of a quarter of a century over *P. cordovanum* and of nearly a century over *P. rovirosae*.

No other species closely comparable with the Brazilian *P. amplum* or *P. rohrii* having been found so far in Mexico, it is not improbable that *P. rohrii crassum*, collected only once in the state of Oaxaca, not far from Chinantla, is a broader-leaved form of *Piper chinantlense*.

## II. PIPER JALAPENSE

C. DECANDOLLE (7) in a posthumously published paper in 1920 named and described a plant as follows:

"*Piper botteri* C. DC., n. sp.—Ramulis glabris; foliis magnis longiuscule petiolatis, limbo oblongo-elliptico basi leviter inaequilatera breviter cordato apice breviter et acute acuminato, supra glabro subtus ad nervum centralem longe piloso et ad laterales breviter hirtello, nervo centrali nervos arcuato-adscendentes utrin-

que 4-5 emittente quorum supremus paullo supra medium centralis solutus, nervis ejus lateralibus utrinque 2-3 utrinque a basi solutis, petiolo usque ad limbum vaginante dorso pilis longis haud densis adpresse munito; pedunculo quam petiolus multo brevior glabro, spica florente quam limbus 4-plo brevior sat crassa et apice longe mucronata, bracteae vertice inflexo triangulari margine subglabro pedicello lato utrinque villosa et dorso subproducta, antheris ovatis quam filamenta brevioribus, ovario libero subtetragono glabro, stigmatibus linearibus."

"Ramuli spiciferi 5 mm. crassi. Limbi in sicco membranacei minute pellucido-punctulati, usque ad 27 cm. longi 13 lati. Petioli 7 cm. Pedunculi fere 1 cm. longi. Spica 5 mm. crassa, ejus mucro 1 cm. longus, stamina 4, stigmata 3 sessilia."

"Orizaba (Botteri et Sumichrast n. 1526, h. Van Heurck.)."

In 1869 C. DE CANDOLLE (4) in the *Prodromus* had described *Piper novae-hispaniae* in almost the same terms, as the following quotation will show:

"*P. novae-hispaniae*.—Foliis petiolatis late suboblongo-ellipticis apice breviter acuminatis, acumine acuto, basi inaequali cordatis supra glabris basi ad nervum centalem adpresse hirtis subtus ad nervos nervulosque hirsutis membranaceis subopacis subtilissime pellucido-punctulatis, nervis subtus subprominulis, centrali ad  $1/2$ - $2/3$  longitudinis nervos alternos subadscendentes utrinque 7 supremos ad apicem ductos mittente, petiolo dorso hirtello ad limbi basin usque vaginante, pedunculo glabro quam petiolus triplo brevior, amento florente quam folium multoties brevior cylindrico mucronato, bracteae cucullatae basi dorso hirsutae vertice inflexo peltata triangularem simulante. In Nova Hispania (Pav. in h. Boiss.). Ramuli glabri. Limbi 0,16 longi, 0,09 lati. Petioli 0,025 longi. (v.s.)"

MARTENS and GALEOTTI in 1843 (18) had given essentially the same description to their *Piper jalapense*.

"*Piper jalapense*, Nobis. (Coll. H. Gal. no. 6012).—Caule fruticoso glabro, foliis alternis venoso-nervosis ovato-oblongis acuminatis basi inaequaliter subcordatis supra laevigatis subtus in nervis ac venis pilosiusculis, spicis breve pedunculatis oppositifoliis petiolo sesquialongioribus. Folia 7-8 pollices longa, 4-5 pollices lata, petioli  $1\frac{1}{2}$  pollicares alati amplexicaules."

"Croît dans les bois humides et au bord des ruisseaux de Xalapa et de Mirador, de 3,000 pieds à 4,500 pieds. Fl. vertes. Juin."

KUNTH (16) in 1839 had already described the same plant when he spoke of *Schilleria lapathifolia*, although his description was based on a specimen collected by SCHIEDE.

"*Schilleria lapathifolia*.—Ramulis glabris; foliis suboblique ellipticis, breviter acuminatis, basi inaequaliter cordatis, membranaceis, pellucide glanduloso-punctulatis, supra glabris et nitidis, subtus in nervo, venis et venulis hirto-pubescentibus; petiolis utrinque membranaceo-alatis, dorso pilosis, pedunculum triplo superantibus; spicis erectis, rectis, folio multoties brevioribus; bracteis cucullatis, margine, ciliatis; ovariis inferne connatis?; stigmatibus 3, recurvatis, fructibus. . . ."

"*Piper macrophyllum* Cham. et Schlecht. in Linnaea 5:73. (excl. synon.)."

"Crescit in sylvis prope Jalapam Mexicanorum."

"Ramuli flexuosi, exsiccati angulat, glabri. Folia alterna, petiolata suboblique elliptica, breviter acuminata, basi inaequaliter cordata, reticulato-venosa, venis primariis utrinque 7-8 nervoque medio subtus prominentibus, infimis parum approximatis, membranacea, subtilissime glanduloso-punctulatis[sic!], glandulis croceis, pellucidis, supra glabra et nitida, subtus pallidiora inque nervo, venis et venulis hirto-pubescentia, 10-pollicaria, 5 pollices lata. Petioli  $1\frac{1}{2}$ -2-pollicares, utroque margine membranaceo-alati, dorso pilosi. Spicae oppositifoliae, solitariae, erectae, rectae, cylindraceae, mucronatae, subbipollicares, crassitie pennae gallinaceae; pedunculo 4-6 lineas longo, glabro. Flores densi. Bracteae cucullatae, subtriangulares, carnosae, glabrae, margine ciliatae. Stamina quot?. Filamenta filiformia, fusca, glabra, persistentia. Antherae reniformes, biloculares. Ovaria abbreviata, glabra, inferne connata. Stigmata 3, sessilia, elongata, crassiuscula, recurvata. Fructus. . . Praecedenti [*Schilleria aurita*] valde affinis."

STEUDEL (23) in 1841 used the species name *lapathifolium* of KUNTH, but, as was his custom, put the species under the genus *Piper*.

Apparently neither C. DE CANDOLLE in describing *Piper botteri*, nor MARTENS and GALEOTTI in describing their *P. jalapense*, identi-

fied their specimens with the descriptions in the earlier publications of STEUDEL or KUNTH, but since all the descriptions are identical in essentials these are one and the same species, which should be known by the name of *P. lapathifolium* (Kunth) Steud., the first name given to it under *Piper*.



FIGS. 2, 3.—Fig. 2: *a*, *P. jalapense* Steud. (Galeotti, Kew), collected near Jalapa; *b*, *P. oblongum* Mart. & Gal. (Galeotti 6009, Brussels), collected near Jalapa; fig. 3, *c*, *P. amalago subpanduriforme* Trelease, from leaf of type specimen in Vienna Herbarium, part of which is now in University of Illinois Herbarium (Wulfschlaegel 130); *d*, *P. amalago* Linn. from photograph now in University of Illinois Herbarium, of type specimen in Linnaean Herbarium; *e*, *P. medium* Jacq., from tracing of sketch in *Icones Plantarum Rariorum* v. 1. p. 2. t. 8 (1781) 93; *f*, *P. longum arboreum*, etc. Sloane, from notes of Trelease (taken from publications of Sloane and Plukenett (21), (22); *g*, *P. amalago* Pluk. (also taken from same publications).

*Piper jalapense* C. DC., which is the *Artanthe jalapensis* Miq. (based on Gal. 6009), is not the same as *P. jalapense* Mart. and Gal. (based on Gal. 6012), as the following description by MIQUEL (20) will show if it is compared with that given by MARTENS and GALEOTTI (fig. 2a):

“*Artanthe jalapensis*.—Ramulis petiolisque hispidulo-hirtis, foliis

rigido-membranaceis obsoletissime pellucido-punctulatis, supra opacis verrucis et pilis rigidis sensim deciduis asperis, subtus pallidioribus epunctatis in nervo costis venulisque pilis mollibus dense pubescentibus, oblongis vel obovato-oblongis, versus basin attenuatam inaequilateris, basi ipsa inaequali obtusiusculis, apice suboblique acuminatis, costis utrinque 6 rarissime 7, amentis cylindricis, rectiusculis mucronatis folio brevioribus, pedunculo petiolum superante, bracteis peltatis pelta suborbiculari fere tota hirtociliata."

"Habitat in m. Cordilleras ad 4,000 p. alt., prope Jalapa Regni mexicani (Gal.! 6009 in h. DeLess.)."

"Frutex *A. asperifoliae* nimis fortasse propinquus sed quantum ex unico ramulo dijudicandum, foliorum forma oblonga vel obovato-oblonga, versus basin attenuata, pilis subtus densioribus et mollioribus, punctorum nigrorum defectu, amentis longius pedunculatis pro diverso habendus. Ramulus in sicco subtetragonus patentim et implexe hirtus, sensim glabratus et asperior, internodiis 3-4½ cent. longis. Folia patentia, rigido membranacea non nisi ad lentem sparsissime pellucido-punctulata, supra saturate viridis opaca, verrucis albis rigidis pilisque verrucis insertis sursum directis sensim deciduis asperrimis admodum aspere subtus glaucescentipallida, punctis nigris haud notata, in nervo costis venulisque pilis mollibus appressis dense puberula, nascentia fere sericea, petiolis hirtis et antice canaliculatis 1-1½ cent. longis sustenta, oblique oblonga et obovato-oblonga, inaequilatera, pleraque versus basin contracta, basi ipsa inaequali obtusa, raro in summis acutiuscula, apice longius acuminata, acumine acuto, marginibus subrepandis planiscula, 13-19 cent. longa, 4-7 lata; e nervo medio percurrente utrinque usque ad ½ circiter alt. 6, rarissime 7 costa; exceptis infimis tenuioribus, alte versus et juxta margines adscendentes oriuntur, quarum summa in acumen nec tamen ad ultimum apicem ducitur. Anastomoses subhorizontales parallelae prominentes. Stipula oppositifolia lanceolata naviculares curvula puberula 1½ cent. longa. Amentum submaturum erectopatulum, pedunculo hirtello fere 2 cent. longo sustentum, leviter curvatum, cylindricum apice breviter mucronatum, 8 cent. longum 3 mm. propemodum crassum, adspectu griseum. Flores annulati. Bractea pedicellata peltata pelta subrotunda, area per-

exigua centrali nuda caeterum hirto-ciliata pilis sordide flavis. Antherae effoetae castaneo-fuscae reniformes (sec. sp. cit.)."

Since these plants are not the same, and since *Piper jalapense* Mart. and Gal. was named prior to *P. jalapense* (Miq.) C. DC., even though as shown above it as well as *P. botteri* appears to be synonymous with *P. lapathifolium*, according to the Neo-American code the name *jalapense* is not applicable in the latter case. Hence the name **P. pseudo-jalapense** nom. nov. may be used to designate *P. jalapense* (Miq.) C. DC.

In the Brussels Herbarium, under the number Galeotti 6009, there are two separate and distinct species, one the foregoing *P. jalapense* C. DC., which is a scabrous and hairy plant, the other a smooth and smaller-leaved almost entirely glabrous species, which MARTENS and GALEOTTI (18) believed to be *P. oblongum* H. B. K. (15) (fig. 2b).

It chanced that two separate and distinct Mexican species have been called *Piper oblongum* H. B. K., which is a Venezuelan plant (Willd. Herb. no. 671). CHAMISSE and SCHLECHTENDAL (2) referred a Mexican plant to *P. oblongum*, but KUNTH (16), the author of the Venezuelan species, nine years later made a new species of it, calling it *Steffensia oblonga*, in contrast to his *Schilleria oblonga* which is the true *P. oblongum* H. B. K. MIQUEL (20) held the same view in 1844, when he called the former *Artanthe chamissonis* and the latter *A. oblonga*. C. DE CANDOLLE (4) in 1869 used MIQUEL's specific names with the generic name *Piper*, to designate these two plants. It will not be necessary, therefore, to consider *P. oblongum* Cham. and Schlecht.

The following description of *Piper oblongum* H. B. K. by KUNTH is taken from *Linnaea*, vol. 13, p. 696, because it is more complete than that first given by KUNTH in H.B.K.

"*Schilleria oblonga*.—Ramulis glabris; junioribus obsolete tuberculatis; foliis breviter petiolatis, elliptico-oblongis, acuminatis, basi aequalibus vel inaequalibus ibique acutis vel obtusis, membranaceis, obsolete pellucido-punctulatis, opacis, glabris, subtus in nervo et venis obsolete puberulis; spicis rectis, folio 1/3 brevioribus; pedunculo petiolum subaequante; bracteis inflexo-peltatis, ciliato-villosis; fructibus obovato-turbinatis trigonis; semine laevi."

"*Piper oblongum* Humb. et Kunth, Nov. Gen. 1:52. Willd. Herb.

no. 671 (fragmentum). *Piper secundum* Willd. Herb. No. 647 majore parte (excl. synonym.)."

"Crescit in Nova Andalusia, prope Cumanacoam."

"Rami nodosi, teretiusculi, exsiccati sulcati, glabri; internodiis  $1\frac{3}{4}$ -2 pollicaribus, ramuli juniores obsolete tuberculati; internodiis  $\frac{1}{2}$ -1 pollicaribus. Folia alterna, breviter petiolata, elliptico-oblonga, acuminata, acumine acuto, basi inaequalia vel aequalia ibique acuta vel obtusa, reticulato-venosa, venis utrinque 4, subparallelis nervoque medio subtus prominentibus, membranacea, obsolete pellucido-punctulata; utrinque glabra, opaca, subtus pallidiora inque nervo medio et venis obsolete puberula  $4\frac{3}{4}$ -5-pollicaria, 24-26 lineas lata. Petioli 2-2 $\frac{1}{2}$  lineas longi, glabri. Stipulae. . . . Spicae oppositifoliae, solitariae, pedunculatae, rectae, cylindraceae,  $3\frac{1}{4}$  poll. longae; fructiferae crassitie pennae columbinae; pedunculo 3 lineas longo, glabro. Fructus densi, sessiles, solubiles, bracteis interpositis totidem inflexopeltatis, vertice triangularibus ibique margine ciliato-villosis, obovato-turbinati, trigoni, apice rotundato-truncati, stigmatibus tribus persistentibus coronati, glabri, magnitudine grani *Papaveris dubii*. Semen fructi conforme obovatum, trigonum, laeviusculum, castaneofuscum, opacum(?)."

"*Piper oblongum* Schlechtend. et Cham. in *Linnaea* 5:73. (excl. synonym.) ex Mexico est *Steffensia oblonga* Kth." (16).

As previously mentioned, MARTENS and GALEOTTI identified their Mexican plant with the Venezuelan species. In addition to the wide geographical break in the habitat of the two species, there is a difference in the form of the plants. TRELEASE, while visiting the Brussels Herbarium, made the following description of the smooth *Piper oblongum* of MARTENS and GALEOTTI (Gal. 6009 in part).

"Stem glabrous; leaves unequally lance-oblong, rather long-acuminate, rather rounded at the base with one side subacute and slightly longer, moderate (scarcely  $5.5 \times 1.3-1.5$ ), pinnately veined from below the upper or middle third, veins  $4-6 \times 2$ , glabrous and rather glossy above, somewhat pubescent on the nerves beneath; petiole short (5 plus 2 mm.), not winged, slightly pubescent; spikes opposite the leaves, moderate (2-70 mm.); peduncle rather short (1 cm.), glabrate; bracts round-subpeltate, ciliate; flowers sessile,

perfect. Type locality: Jalapa, Vera Cruz. Distribution: Eastern Sierra Madre, Mexico."

A critical comparison of these two descriptions of the specimens comprised under Galeotti 6009 proves that two separate and distinct plants are under discussion. Since the name *Piper oblongum* applies to another species, the MARTENS and GALEOTTI plant (Gal. 6009 in part at Brussels) may be known as *P. pseudo-oblongum*, nom. nov., with the foregoing description.

In the sense here used *Piper lapathifolium* is represented in the following principal collections of the world: Jalapa, Vera Cruz (Pringle 8067, Berlin, Field Museum, U.S. Nat. Herb., New York Bot. Gard.; Endlich 1412, Berlin; Galeotti 6012, Paris, Delessert Herb.; Schiede 2, Berlin): near Orizaba (Botteri and Sumichrast 1526, Van Heurck Herb.): Nova Hispania (Pavon in Herb. Boiss.).

*Piper pseudo-oblongum* is represented by Galeotti 6009 in part, at Brussels, collected near Jalapa and Mirador, Vera Cruz.

*Piper pseudo-jalapense* is represented by the collections of GALEOTTI and PRINGLE; near Jalapa, Vera Cruz (Pringle 8074, New York Bot. Gard., U.S. Nat. Herb., Brussels, Field Museum, Kew) (Galeotti at Kew, 6009 in part at Brussels).

A study of the descriptions of *Piper botteri* C. DC., *P. jalapense* Mart. et Gal., and *P. novae-hispaniae* C. DC. proves these species to be synonymous with *P. lapathifolium* (Kunth) Steud. Since the name *P. jalapense* was used by MARTENS and GALEOTTI in 1843, according to the Neo-American code it would not be available for use again, hence MIQUEL's *Artanthe jalapense*, which is synonymous with C. DE CANDOLLE's *P. jalapense*, may be known by the new name suggested above, *P. pseudo-jalapense*. The *P. oblongum* of MARTENS and GALEOTTI, which is part of the Galeotti 6009 at Brussels, is not synonymous with *P. oblongum* of Humboldt, Bonpland, and Kunth, and hence cannot be known by that name; but it may be called, as stated previously, *P. pseudo-oblongum*.

### III. PIPER AMALAGO SUBPANDURIFORME

While WULLSCHLAEGEL, a missionary, was stationed at Fairfield, Springfield, and Chatam, in the parish of Manchester, Jamaica, he collected a number of plants, one of which was a *Piper*, the only one



of its kind ever reported (fig. 3c). In 1853 MIQUEL (19) named and described this plant in the following manner:

"*Enckea subpanduraeformis*.—Foliis supra partem unam quartam quintamve obovatam vel subdeltoideam abrupte utrinque in sinum coarctatis indeque longe lineari-lanceolatis, basi inaequali obtusa tenuiter quintuplinerviis, in parte lanceolata transverse tenere multinerviis, submembranaceis, glabris, nascentibus tenerrimis quibusdam pilis subtus inspersis, amentis pedunculo glabro petiolum superante suffultis, rectiusculis, densiuscule puberis, folio brevioribus, subdensifloris, bracteis conchaeformibus sursum glabris, ovario ovato glabro, stigmatibus 3 brevissimis (albidulis)."

"In Jamaicae insulae umbrosis detexit vincerat. WULLSCHLAEGEL, S. Fidei Evangelicae Missionarius. Vidi in Herb. Martii."

"Habitu, ramificatione scil. dichotoma, ramis ramulisque valde nodosis, nodis perfacile separabilibus, internodiis tenuiter longitrorse striulatis, stipulis parvulis oppositifoliis curvulis hic illic persistentibus, amentorumque fabrica congeneribus suis quam simillima species, *E. discolori* aliisque ex angusta foliatarum phalange proxima, longe a totius ordinis norma discedere videtur foliis prope basin sinu utrinque subpanduraeformiter excisis, fere ad trilobatam formam enitentibus, parte scil. infra sinum sublobato-exserta brevem lobulum utrinque exhibente, terminalem longissimum angustatumque fulciente. Petioli 1–2 lin., folia 2–3 poll. longa, basi 2–6 lin., superne  $1\frac{1}{2}$ – $2\frac{1}{2}$  lin. lata, discolora, supra siccitate subnigrescentia, subtus fuscule-viridula, impunctata, basi leviter inaequalia, sursum attenuata, marginibus subrepanda. Amenta cum pedunculo circiter  $1\frac{1}{2}$ -pollicaria, juvenilia densiflora subflaccida, adultiora magis stricta, recta, erecto-patula. Stamina 4 subsessilia, ovario regulariter circumposita, antheris fusco-luteis cordatis, loculis connectivo nunc nigrescente diremtis, in fructu vix omnino persistentia."

In the *Prodromus* C. DECANDOLLE (4) replaced the name *Enckea subpanduraeformis* by *Piper panduratum*, but later (8) used the specific name given by MIQUEL (changed in spelling by replacement of *i* for *ae*), retaining the generic name *Piper*, since he did not admit *Enckea* as a separate genus. GRISEBACH (12), however, six years after MIQUEL's publication, believed WULLSCHLAEGEL's specimen to be a form of *Piper amalago*, and refers to it as *P. amalago variifolia*

Miq. The "Miq." probably refers to MIQUEL's *Enckea subpanduraeformis*; but no reference can be found indicating that MIQUEL had called it a variety of *P. amalago*, or had applied to it the trivial name *variifolia*. FAWCETT and RENDLE (11) in 1914 used GRISEBACH's name, substituting, as is customary, the generic name *Piper* for *Enckea*. In a recent publication TRELEASE (24) refers incidentally to it as *P. amalago subpanduriforme*.

WULLSCHLAEGEL collected another specimen of *Piper* between Fairfield and Nazareth, which is practically the same locality in which he found the form previously described. C. DECANDOLLE (4) named and described this plant as follows:

"*Piper wulfschlaegelii*.—Foliis breviter petiolatis lanceolatis subovato-lanceotis [sic!] apice acuminatis, acumine acuto, basi aequali acutis utrinque glabris siccis rigido-coriaceis subopacis pellucido-punctulatis 5-nerviis, centrali nervo ad apicem ducto proximis alte supra medium ultimis subtilioribus sub medium ductis, petiolo glabro basi vaginante, pedunculo glabro petiolum paullo superante, amento densifloro folium fere duplo superante, rhachi pubescente, bractea ovario proxima ovato-subrotunda intus et dorso pubescente, staminibus 5-6, ovario glabro, stigmatibus 3. In Jamaica (Wulfschlaegel! in h. Monac.) Rami ramulique glabri parche lenticellosi. Limbi 0,06 longi, 0,02 lati, petioli 0,04 longi. (v.s.)"

A comparison of *Piper wulfschlaegelii* and *Enckea subpanduraeformis* shows the two to be alike except in shape of leaf, the former entire, the latter lobed. The general dimensions are about equal. It would seem therefore that *Enckea subpanduraeformis* might be a form of *Piper wulfschlaegelii*. On the other hand, if we compare *P. wulfschlaegelii* with *P. amalago*, it is practically impossible to differentiate between them except for the slightly narrower leaves of the former. *P. amalago* was described by LINNAEUS (17) as follows:

"*Piper foliis lanceolato-ovatis quinquenerviis rugosis. Amalago.*"

Even from the more complete description given by C. DECANDOLLE (8), no other essential difference can be noted:

"*Piper amalago* Linn. foliis modice petiolatis, e basi ima acuilatera et acuta ovatis vel ovato-ellipticis, apice longiuscule acuminatis acumine acuto mucronulatoque, utrinque glabris, 5-7-nerviis, petiolo basi ima vaginante pedunculoque petiolum paullo superante

glabris; spica limbum aequante vel eo paullo brevior densiflora glabra, bracteae ovario proximae obovato-subrotundae glabrae vertice inflexo; staminibus 5-6; bacca ovata glabra. . . ."

"Frutex ramulis glabris, spiciferis in sicco  $1\frac{1}{2}$  mm. crassis, collenchymate haud libriformi in fasciculos discretos disposito fasciculis intramedullaribus 1-seriatis canali vacuo nullo. Limbi in sicco membranacei, subpellucidi, creberrime pellucido-punctati, 9-12 cm. longi et 4-6 cm. lati; petioli superiores adulti usque ad 1 cm. longi. Pedunculi circiter  $1\frac{1}{2}$  cm. longi; rhachis glabra. Stamina rhachi inserta, antheris reniformibus. Bacca vix  $1\frac{1}{2}$  mm. longa, stigmatibus ovatis crassiusculis et brevibus coronata."

"Hab. in Jamaica: P. Browne, Swartz (in H. DC. et in H. Willd. n. 684), Sloane, Distin, Alexander (in Herb. Kew), Millspaugh n. 1943." (fig. 3d).

Another later recognized Jamaican species, *Piper nigrinodum*, collected near Troy (Harris 9075), has nearly the same characteristics as the species already named, as the following description will show (9):

"*Piper nigrinodum* C. DC. (n. sp. e sect. Enckea).—Foliis breviter petiolatis, ovatis, basi subaequilatera rotundatis, apice acuminatis acumine obtusiusculo, utrinque glabris, 5-nerviis petiolo basi ima vaginante; pedunculo petiolum fere duplo superante, glabro, spica folii limbum paullo superante, subdensiflora; bractea rotundata inferne dorso hirtella, basi late truncata, rhachi puberula; antheris rotundato-reniformibus, filamentis brevissimis; ovario obconico, glabro, apice stilo carnosio umbonato munito; stigmatibus brevissimis, glabris; bacca elliptica, glabra, apice stilo carnosio umbonata, subacuta."

"Black-jointer incolarum."

"Frutex 10 ped. altus. Ramuli levissime costulati nodique glabri, in sicco nigri; spiciferi 1 mm. crassi, in 2 mm. crassis collenchyma haud libriforme in fasciculos discretos dispositum, fasciculi intramedullares 1-seriati canalis vacuus nullus. Limbi in sicco membranacei, creberrime pellucido-punctulati, usque ad 7 cm. longi; et 38 mm. lati; petioli 5 mm. longi. Pedunculi 12 mm. longi; spicae bacciferae circiter 8 cm. longae. Stamina 4-5. Baccae fere 2 mm. longae."

"Hab. in Jamaica prope Troy, alt. 500 m., m. Nov. Fruct.: W. Harris 9075 (H. Berol.)." (fig. 3e).

Material believed to be representative of *Piper nigrinodum* collected by MAXON and KILLIP no. 1504, near Ipswich, St. Elizabeth, shows leaf sizes from  $2 \times 5.5$  to  $4 \times 8$  cm. A photograph in the Herbarium of the University of Illinois, made for the secretary of the Linnean Society from the type specimen of *P. amalago* in the British Museum at South Kensington, England, shows leaf variations between  $2.5 \times 6$  cm. and  $4 \times 8.5$  cm. This seems to warrant the conclusion that, so far as leaf size and shape are concerned, which are the only essential differences, *P. amalago* may be held to include *P. nigrinodum* and *P. wulfschlaegelii*.

*Piper amalago subpanduriforme*, as shown by a few twigs and leaves in the University of Illinois Herbarium, taken from part of the original collection in the Vienna Natural History Museum, has the black stems and joints of the "Black-jointer," *P. nigrinodum*. This at least shows some relation within the group, whether it is important or not.

In 1825 HAMILTON (13) described a glabrous, 3-5-nerved Jamaican *Piper* under the name *P. cellidifolium*, apparently unaware that this name had been applied ten years before to a Venezuelan *Piper*. DIETRICH (10), however, recognizing the Venezuelan form as *P. cellidifolium*, called the Jamaican form *P. dubium*. HAMILTON's description shows that his *P. cellidifolium* will readily fall within the *amalago* group:

"*Piper cellidifolium*.—Fol. 3-5-nerviis alternis oblique cordatis lanceolato-acutis margine tenuissime cartilagineis glaberrimis; spad. oppositifoliis. *P. celtidifolium*. Herb. Prof. Desv. Antilles. (s.v.)"

Another Jamaican specimen (March 1403 in Griseb. Herb.) has been referred to in URBAN's *Symbolae Antillanae* (8) as *Piper richardianum*, but it is likely that on reexamination it may prove to be *P. amalago*, since the characteristics of the two species are quite similar, and MARCH's specimen is the only "*Piper richardianum*" reported from Jamaica.

The Jamaican specimens of *Piper amalago*, *P. nigrinodum*, *P. wulfschlaegelii*, *P. dubium*, and *P. amalago subpanduriforme* are described as glabrous, except for the rachis, which is pubescent. On the other hand is *P. medium*, which is pubescent to hairy on the petiole and the nerves on the lower side of the leaf, and has broader leaves with rarely or never an unguiculate base. *P. medium* was

named and described by JACQUIN in *Icones Plantarum Rariorum* (1: 2, t. 8. 1781-93). Since his description is very brief, the following description of *P. medium* as understood by C. DECANDOLLE is taken from his *Prodromus* (4).

*P. medium* (Jacq. ic. rar. v. 1. p. 2. t. 8.), foliis petiolatis elliptico-lanceolatis vel ovato-lanceolatis apice acuminatis, acumine obtusiusculo mucronulato, inferioribus basi aequali cordatis super. basi. aequali rotundatis vel subattenuato-acutiusculis vel basi ima subcuneatis cuneatisve supra glabris subtus ad nervos puberulis vel glabratissimis siccis membranaceis subpellucidis subtilissime pellucido-punctatis 7-nerviis, nervis supra subprominulis subtus prominulis centrali ad apicem ducto proximis fere ad apicem sequentibus supra medium ductis infimis subtilioribus saepe obsoletis, petiolo basi ima vaginante hirtello dein glabrato, pedunculo glabro petiolum aequante, amento filiformi quam folium  $1/3$  brevius densifloro, rhachi puberula, bracteae ovario proximae obovatae dorso villosulae vertice inflexo nudo, stam. 4, filamentis antheras acquantibus, ovario glabro, stigmatibus 3 sessilibus, bacca glabra emersa ovoidea apice attenuata. . . . Rami glabri, ramuli glabri. Limbi 0,11 longi, 0,05-0,06 lati, petioli, 0,008-0,01 longi. Amenta flor. 0,06 longa, 0,0015 crassa. (v.s.)" (fig. 3f).

The first knowledge of *Piper medium* dates from pre-Linnean times, when PLUKENETT (21) wrote of "*Piper frulex* americ. spica longa gracili" (fig. 3g), and SLOANE (22) wrote of "*Piper longum* arboreum altius, folio nervoso minore, spica graciliori & brevius." A little later (1756) BROWNE (1) wrote of "*P. frutescens* diffusum ramis flexilibus geniculatis, foliis ovatis ad petiolum leniter revolutis."

According to the unpublished notes of TRELEASE, made when he examined the specimens on which PLUKENETT based his description (Herb. Sloane Vol. 3. no. 109, South Kensington, England) and SLOANE his description (Herb. Sloane vol. 2. no. 79), the young twigs and petioles of these specimens are slightly velvety to hairy, and the leaves are much larger than the descriptions given above for *P. amalago*. BROWNE's type of leaf is more ovate than that of SLOANE, but the broader leaf and the pubescence let them fall under another group, of which *P. medium* is the type.

There is another important distinction between these two groups

from Jamaica. The leaves of *P. medium* seldom or never have an unguiculate base, while at the leaf base of *P. amalago subpanduriforme* in the Herbarium of the University of Illinois there is quite frequently a slight inrolling or unguiculation. This is to be seen on the leaves also of *P. nigrinodum* in the same herbarium. TRELEASE (24) shows that this is true also of *P. amalago* as commonly understood.

Because of this unguiculate base and because of the general characteristics of the plant, it seems almost certain that some of the Jamaican plants referred to *Piper unguiculatum* (4) have been misidentified with the similar but different South American species of the same name, the stems and leaves of which dry a light green color compared with the blackened stems and leaves of the Jamaican specimens observed. Also, *P. unguiculatum* has been reported only twice outside the South American continent. Once it was collected at the Botanical Garden of Trinidad off the coast of Venezuela and noted as a wild or escaped plant from the Botanical Garden there. Again it was cultivated as a decorative plant at Basse Terre on the Island of Guadeloupe. It is certain, therefore, that Jamaican plants referred to *P. unguiculatum* are merely *P. amalago*.

In the sense here used *Piper amalago* is represented in the following principal collections of the world (exclusively from Jamaica and the Caymans): near Port Antonio (Fredholm 3313, U.S. Nat. Herb.; Hitchcock, Field Museum): near Hope Tavern (Harris 6590, U.S. Nat. Herb., Urban's Herb., Kew): near Stanmore Hill (Harris 9972, U.S. Nat. Herb., Urban's Herb.): near Williams Ford (Millspaugh 1943, Field Museum, Urban's Herb.): near Troy (Harris 9075, N.Y. Bot. Gard., U.S. Nat. Herb., Kew (cotype)): near Potsdam Hill (Harris 9805, U.S. Nat. Herb., Urban's Herb.): near Yallahs Valley (Harris 6505, U.S. Nat. Herb., Urban's Herb.): on the road to Guava Ridge (Harris 8354, Urban's Herb.): at Hope Mines (Harris 8353, Urban's Herb.): near Mendeville (Britton 1027, N.Y. Bot. Gard., U.S. Nat. Herb.): near Green River (Harris 8342, Urban's Herb.): in Blue Mts. (O. Hansen, Copenhagen, Urban's Herb.): near Ginger River (D. S. Johnson 26, Field Museum): near Diabolo (Kew): no locality given (Lloyd 1094; Millspaugh 1299; Rothrock 224; Harris 6505, 8342, 8353, 9075, 9972, Field

Museum; Britton 341, Field Museum; Disdain at Kew (labeled *Enckea cellidifolium*); Nilson 178, Kew; Alexander, Kew; Balbis, Dahlem; Bertero (as *discolor-berteroanum* C. DC.).

In contrast with this, *Piper medium* is represented only by the following: near Toms River (Thompson 8049, Urban's Herb.): no locality given (Schwartz in Herb. Vahl. [labeled as *P. amalago*]; McMurray 626, Herb. DC.).

These facts seem to justify the position taken by GRISEBACH when he held *Enckea subpanduraeformis* Miq. to be a form of *Piper amalago* L. They equally justify the conclusion that *P. wulschlaegelii*, which has been collected only twice, is to be considered as *P. amalago*. On this basis the *amalago* peppers of Jamaica, which are glabrous except for the spike rachis, are to be called *P. amalago* L., with the synonyms *P. wulschlaegelii* C. DC., *P. nigrinodum* C. DC., *P. richardianum* C. DC. (Jamaican forms only), *P. dubium* Diet., and *P. unguiculatum* Ruiz and Pav. (Jamaican forms only), and with *Enckea subpanduraeformis* Miq., a varietal form or monstrosity bearing the name *P. amalago subpanduriforme* (Miq.). Admitting *P. medium* tentatively to the Jamaican flora, we may note that it is represented only by the pubescent specimens previously mentioned, and by the two specimens in the Sloane Herbarium.

<sup>1</sup> HARVARD, ILL.

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## ASPECTS OF THE MADEIRA FLORA

T. D. A. COCKERELL

In less than fifty years, Mr. CHARLES O. L. POWER has developed a garden at Quinta do Deão, Madeira Island, which contains a most remarkable collection of living woody plants that has been too much neglected by botanists.

When we consider any locality from a botanical standpoint, we may distinguish between the actual and possible flora. Thus, for example, a desert area may not have more than a small number of the existing plants which would grow there, but when we have enumerated these, there remain innumerable species which could not survive under the conditions found. As a broad proposition, this is commonplace and uninteresting; but the Madeira Islands afford such an extreme case of divergence between the indigenous and potential flora that they are worthy of study on this account.

The mean annual temperature at Funchal, according to LOWE, is 65°-67° F.; that of Porto Santo, observed by Mr. A. C. DE NORONHA, is a little higher. The climate is equable, without great extreme, but in the winter or early spring one may see snow on the high mountains above Funchal. The native flora is strictly Palaearctic, the few apparent exceptions probably representing types which formerly existed in Europe, but which are now extinct there. Nevertheless, Mr. POWER's garden has an essentially tropical aspect, and there is no doubt that the lower levels on the island are suited for many kinds of tropical vegetation. What the limitations are we do not yet know, but plants which require extreme heat to ripen their seeds could not be perpetuated (unless vegetatively) in the wild state. Thus the date palm, which grows luxuriantly, fails to produce edible fruit.

A very good catalogue of the flora of the Madeira Islands was published at Funchal by C. A. DE MENEZES in 1914. He lists in all 951 species of flowering plants and vascular cryptogams. Of these, 111 are marked with two asterisks, as being widely cultivated forms, not properly belonging to the flora. No less than 154 others are known to be introduced species which have become established. This

leaves only 686 possibly native, and there is no doubt that many of these have been introduced during the long period of human occupation. In the case of *Eriobotrya japonica* (Thunb.) Lindl., the commonly cultivated and much appreciated loquat, the asterisks have been omitted by accident. It has been estimated that there are from 80 to 90 species of endemic flowering plants, and about 110 peculiar to Madeira and one or other of the Atlantic islands. The exact number of genuine endemics will not be known until further revisions are made, but it is certain that the number is quite large, perhaps not less than one-fifth of the really indigenous flora. The Azores (TRELEASE, 1897) have only 39 species of endemic flowering plants and vascular cryptogams, but their topography is less suitable for a large flora.

Enumerating the endemic plants of the Madeiras, we find two classes: those which closely resemble European species, and those which are quite distinct and isolated. The latter, mainly woody plants, represent the older flora, and, like the endemic snails, must have arrived far back in Tertiary time. The first of these groups includes such species as *Sambucus maderensis* Lowe, once considered a variety of *S. nigra*; *Sorbus maderensis* (Lowe) Dode, described as a variety of *S. aucuparia*; and *Ruscus* (? var. of *hypophyllum* L.) *lanceolatus* (Lowe). The second group, of which several species are well figured in *Die Vegetation der Erde*, IX, Bd. 1, consists of such as the following, if we include in it species peculiar to the Atlantic islands, but not necessarily to the Madeiras:

*Pilosporum coriaceum* Aiton.—Endemic, extinct, or nearly so. Species of *Pilosporum* occur in the European Oligocene and Miocene.

*Visnea mocanera* L. f.—Madeira and Canaries only.

*Monizia edulis* Lowe. —A remarkable umbellifer, also on the Savage Islands.

*Clethra arborea* Aiton.—Endemic species of an American genus. *Clethra* also occurs (well preserved fruits) in Baltic amber, so it is not necessary to suppose that the ancestor of *C. arborea* came direct from America.

*Heberdenia excelsa* (Aiton) Banks.—Madeira and Canaries only. Often referred to *Ardisia*, of which there are several European Tertiary species.

*Sideroxylon marmulano* Lowe.—Endemic, now very rare. The genus occurs in the European Tertiary rocks.

*Notelaea excelsa* (Aiton) Webb.—Madeira, Canaries, and Azores only. SAPORTA described a species from the Pliocene.

*Phoebe indica* (L.) Pax., also called *Persea indica* (L.) Spr.—Madeira and Canaries only. The Azores have an endemic *Persea azorica* Seub., and the genus occurs in the European Miocene.

*Apollonias barbusana* (Cav.) W. & B., or *A. canariensis* (Willd.) Nees.—Madeira and Canaries only.

*Ocotea foetens* (Aiton) B. & H.—Madeiras and Canaries only.

*Dracaena draco* L.—Madeiras and Canaries only. SAPORTA described species, ascribed to this genus, from the Eocene and Oligocene of Europe. The genus is well represented in Africa today.

Thus we have preserved in the Atlantic islands remnants of the Tertiary flora of Europe, modified of course as to species.<sup>1</sup> The snails of the Madeiras show just the same sort of thing.

On the other hand, not only is the tropical or subtropical flora wholly absent from the islands (except as introduced), but many prominent Palaearctic genera are lacking, such as *Acer*, *Alnus*, *Betula*, *Carpinus*, *Cornus*, *Corylus*, *Fagus*, *Fraxinus*, *Pinus*, *Prunus*, *Quercus*, *Ribes*, *Tilia*, *Ulmus*, *Vitis*, etc. There are no palms or tree-ferns.

It is of course possible to postulate a considerable extinct flora, and to suppose that many species were destroyed in quite recent times by the burning of the forests or through the intensive cultivation of the mountain sides and valleys and the introduction of weeds. I myself noticed, in the vicinity of the Portella Pass, how the people burned over the *Ilex*-covered hillsides. One is fascinated by the thought that remarkable species may still lurk, nearly extinct, on steep cliffs or in deep valleys of the mountains.

Many years ago a deposit of fossil plants was found in the São Jorge Valley, Madeira, and in 1857 HEER published a list of 26 species, of which 18 were definitely determined. Seven were treated as new, and placed in the genera *Asplenium*, *Salix*, *Corylus*, *Ilex*,

<sup>1</sup> According to G. DEPAPE, the actual species *Ilex canariensis*, *Persea* or *Phoebe indica*, and *Oreodaphne* or *Ocotea foetens*, now living in Madeira, occur fossil in the Pliocene of the Rhone Valley. With these occur *Liriodendron tulipifera*, *Sassafras*, *Ginkgo*, *Torreya*, *Sequoia*, etc.

*Pistacia*, and *Phyllites* (? *Rhus*). One species was doubtfully referred to *Ulmus suberosa*. The flora was presumably not earlier than Pleistocene, but still of considerable interest, as it indicated several extinct species. LOWE, however, who knew the Madeira flora better than any one else, declared that the supposed new *Ilex* was *Vaccinium maderense*, the supposed *Ulmus* and the *Corylus* were leaflets of the native *Rubus*, the *Pistacia* was not of that genus, and so forth. Thus, as the matter stands, we are not certain that we know any extinct species. Unfortunately it is no longer possible to find any fossil plants at this place. Other similar remains were reported from Porto da Cruz, on the north shore of the island. I went there in search of them, but could find nothing recognizable.

There is a depth of 2400 fathoms between Madeira and the Canaries, of 2225 between Madeira and the African coast, of 2675 between Madeira and the Azores. The accumulated evidence of all sorts goes to show that the Madeiras are truly oceanic, and were never united with the mainland. WALLACE (Island Life, 2d. ed., 1892), in discussing the Azores, has gone rather fully into the question of transportation across the sea. J. Y. JOHNSON listed no less than 70 species of birds as visitors or stragglers to Madeira, and these must have been instrumental in bringing seeds. The birds in question are Palaearctic species, including some Mediterranean types. The trade winds blow southward from the direction of the Spanish peninsula, and the Canaries current has the same general direction. Thus floating objects might come from the west of Europe, or even across the Atlantic, but not from the south, and hardly from the African coast.

In Mr. POWER's garden are 56 species of palms, 9 of cycads, 12 of tree-ferns, the last practically all grown from spores, except *Cyathea dealbata*. Most of the plants were grown from seed, as it is very difficult as a rule to import living plants. Mr. POWER not only gave me a list of the principal species, but took me around the garden, and I personally examined nearly all of them, and found them well developed and in good condition. Allowing for the favorable conditions of cultivation, it must be admitted that the soil and climate of the island are adapted for an amazing diversity of plants,

so that the insular nature of the actually native flora becomes more strikingly evident.

I have not undertaken to enumerate all the species in POWER'S collection, but only enough to bring out clearly the point of the argument. I have arranged them according to the region or continent of their origin:

1. AUSTRALIA.—*Alsophila australis* R. Br.; *A. excelsa* R. Br.; *Dicksonia antarctica* Labill.; *Macrozamia moorei* F. Muell.; *Livistona australis* Mart.; *Areca aliciae* W. Hill; *Araucaria bidwilli* Hook.; *Brassaia actinophylla* F. Muell.; *Castanospermum australe* A. Cunn. & Fraser; *Casuarina suberosa* Otto & Dietr.; *C. cunninghamiana* Miq.; *Doryanthes excelsa* Correa; *D. palmeri* W. Hill; *Dammara robusta* C. Moore; *Eucalyptus ficifolia* F. Muell.; *E. globulus* Labill.; *E. amygdalina* Labill.; *E. calophylla* R. Br.; *E. citriodora* Hook.; *E. colossea* F. Muell. (immense tree, from seed); *Ficus cunninghamii* Miq.; *Grevillea robusta* A. Cunn.; *Hymenosporum flavum* F. Muell.; *Lagunaria pattersonii* D. Don. (fine tree); *Randia fitzalanii* F. Muell. (badly infested by the coccid *Saissetia hemisphaerica*, with much black fungus); *Syncarpia laurifolia* Tenore; *Sterculia diversifolia* G. Don.; *S. acerifolia* A. Cunn.; *S. rupestris* Benth. (little tree); *Macadamia ternifolia* F. Muell.; *Stenocarpus sinuatus* Endl.; *Buckinghamia* sp.; *Tecoma smithii* W. Wats.

2. NEW ZEALAND.—*Cyathea dealbata* Swartz; *C. medullaris* Swartz; *Dicksonia squarrosa* Swartz; *D. fibrosa* Col.; *Rhopalostylis sapida* (Soland.) H. Wendl. & Drude; *Cordyline australis* Hook. f.

3. PACIFIC ISLANDS.—*Howea belmoreana* (F. Muell.) Becc. (Ld. Howe I.); *H. forsteriana* (F. Muell.) Becc. (Ld. Howe I.); *Hedyscepe canterburyana* (F. Muell.) H. Wendl. & Drude (Ld. Howe I.); *Veitchia joannis* H. Wendl. (Fiji); *Pritchardia martii* H. Wendl.; *P. pacifica* Seem. (Fiji); *Rhopalostylis baueri* (Hook. f.) H. Wendl. & Drude (Norfolk and Chatham Is.); *Araucaria excelsa* R. Bar. (Norfolk I.); *A. cookii* R. Br. (New Caledonia); *Artocarpus cannoni* Van Houtte (Society Is.); *Ficus parcelli* Veitch (with varying leaves); *Hibiscus tricuspis* Banks (tree); *Pothos aureus* Linden (Solomon Is.).

4. CHINA AND JAPAN.—*Trachycarpus excelsus* (Thunb.) H. Wendl.; *Rhapis flabelliformis* L'Her.; *Bambusa aurea* Sieb.; *Camphora camphora* (L.) Karst.; *Diospyros kaki* L. F.; *Gleditschia sinensis*

Lam.; *Trachelospermum jasminoides* (Lindl.) Lem.; *Ginkgo biloba* L.; *Osmanthus fragrans* (Thunb.) Lour.; *Paulownia imperialis* Sieb. & Zucc.; *Musa coccinea* Andr.; *Wistaria chinensis* D. C.

5. MALAYA.—*Cycas circinalis* L.; *Arenga saccharifera* Labill.; *Calamus asperimus* Blume (small); *Caryota rumphiana* Mart.; *C. blancoi* Hort.; *C. mitis* Lour.; *Livistona altissima* Zoll.; *L. hoogen-dorpii* Andre; *Nenga wendlandiana* Scheff.; *Eugenia pendula* D. C.; *Bombax malabaricum* D. C.; *Broussonetia papyrifera* Vent.; *Casuarina equisetifolia* L.; *Michelia champaca* L.

6. INDIAN REGION.—*Phoenix rupicola* T. Anders.; *P. roebelenii* O'Brien; *Caryota urens* L.; *Oncosperma fasciculatum* Thw.; *Wallichia caryotoides* Roxb.; *Alpinia nutans* Rose.; *Bauhinia tomentosa* L.; *B. purpurea* L. (fine tree); *Beaumontia grandiflora* Wall.; *Caesalpinia sappan* L.; *Cinnamomum cinnamomum* (L.) Ckl.; *Eugenia jambos* L.; *E. malaccensis* L.; *Ficus elastica* Roxb.; *F. religiosa* L. (from seed); *F. benghalensis* L.; *Saraca indica* L.; *Lagerstroemia indica* L.; *L. flos-reginae* Retz.; *Pandanus furcatus* Roxb. (from seed); *Melia azedarach* L.; *Solanum auriculatum* Aiton; *Tamarindus indica* L.; *Murraya exotica* L.; *Quisqualis indica* L.; *Thunbergia grandiflora* Roxb.; *T. mysarensis* T. Anders.; *Lonicera hildebrandiana* Coll. & Hemsley.

7. ISLANDS OF INDIAN OCEAN.—*Latania commersonii* Gmel. (as *L. borbonica*, and perhaps was really *Livistona chinensis*); *L. rubra* Jacq. (said to be *commersonii*); *Ilyophorbe amaricaulis* Mart.; *I. verschaffeltii* H. Wendl.

8. MADAGASCAR.—*Chrysalidocarpus lutescens* H. Wendl.; *Dombeya wallichii* (Lindl.) B. & H.; *Buddleia madagascariensis* Lem.; *Cyperus alternifolius* L.; *Ravenala madagascariensis* J. F. Gmel.; *Pandanus utilis* Bory; *Stephanotis floribunda* Brougn.; *Delonix regia* (Bojer) Raf. (has never flowered).

9. AFRICA.—*Phoenix reclinata* Jacq.; *Corypha umbraculifera* L.; *Blighia sapida* Kon.; *Euphorbia candelabrum* Trem.; *Erythrina tomentosa* R. Br.; *Kigelia pinnata* D. C.; *Plumbago capensis* Thunb.; *Schottia speciosa* Jacq.; *Strelitzia reginae* Banks; *Spathodea campanulata* Beauv.; *Asparagus sprengeri* Regel; *Encephalartos lehmanni* Lehm.; *E. cycadifolius* var. *friderici-guilielmi* (Lehm.) Rod.; *E. altensteinii* Lehm.; *E. villosus* Lam.; *E. caffer* Miq.

10. EUROPE AND MEDITERRANEAN REGION.—*Phoenix dactyli-*

*fera* L.; *Cyperus papyrus* L.; *Buxus sempervirens* L.; *Fagus sylvatica* var. *purpurea* Aiton.

11. CANARY IS.—*Phoenix canariensis* Chab.

12. NORTH AMERICA.—*Magnolia grandiflora* L. (two, very fine); *Butneria florida* (L.) Kearney; *Serenoa serrulata* (Michx.) Hook. f.; *Washingtonia filifera* (Linden) H. Wendl.; *Sabal glabra* (Mill.) Sarg.

13. MEXICO AND CENTRAL AMERICA.—*Acanthorrhiza mocinni* (H. B. K.) B. & H.; *Chamaedorea* sp.; *Erythea edulis* (H. Wendl.) S. Wats.; *E. armata* S. Wats.; *Washingtonia sonorae* S. Wats.; *Ageratum mexicanum* Sims.; *Beaucarnea recurvata* Lem.; *Bignonia cherere* Lindl.; *Datura suaveolens* Poir.

14. WEST INDIES.—*Oreodoxa oleracea* Mart.; *Roystonea regia* (H. B. K.) O. F. Cook; *Sabal blackburniana* Glazebrook (slow growing); *Thrinax parviflora* Sw.; *Erythrina speciosa* Andr.; *Hibiscus elatus* Sw. (tree, from seed); *Theophrasta imperialis* Linden.

15. SOUTH AMERICA.—*Cocos plumosa* Hook.; *C. weddelliana* H. Wendl.;<sup>2</sup> *Diplothemium maritimum* Mart.; *Jubaea spectabilis* H. B. K.; *Trithrinax brasiliensis* Mart.; *Brownea coccinea* Jacq.; *B. grandiceps* Jacq.; *B. ariza* Benth.; *Bombax erianthus* Cav. (said to be *Eriodendron leiantherum* D. C.); *Bignonia purpurea* Lodd.; *Bougainvillea glabra* Choisy; *B. spectabilis* Willd.; *B. spectabilis* var. *lateritia* Lem.; *Crescentia cujete* L. (small, from seed); *Cestrum foetidissimum* Jacq. (*nocturnum* L. is the prior name); *Erythrina crista-galli* L.; *Eugenia brasiliensis* Lam.; *Hura crepitans* L.; *Calliandra tweedii* Benth. (as *Inga pulcherrima* Cerv.); *Jacaranda ovalifolia* R. Br.; *Schinus molle* L.; *Parmentiera cereifera* Seem.; *Swietenia mahagoni* Jacq. (small tree); *Solandra grandiflora* Sm.; *Mandevilla suaveolens* Lindl.; *Phytolacca dioica* L.; *Thevetia neriifolia* Juss.; *Streptosolen jamesonii* Miers. (this fine ornamental plant was sent to Madeira from Kew many years ago, and is now seen in gardens everywhere); *Monstera deliciosa* Liebm.

The following conclusions appear to be justified:

1. The Madeira Islands are "oceanic," and have received their indigenous flora from across the water, floating or carried by birds.

2. The means of transport are still in operation, and there are

<sup>2</sup> *Cocos nucifera* L. was in the collection, but it died. Madeira is not tropical enough for the coconut.

many indigenous plants which are unmodified, and others which are so little altered that authors have been divided in the matter of according them specific rank.

3. There are many introduced plants, however, and in the case of such species as *Portulaca oleracea* L., *Papaver rhoeas* L., *Trifolium repens* L., *Anagallis arvensis* L., etc., assumed by MANEZES to be native, it is impossible to be sure whether they were brought by man or came otherwise.

4. There is an ancient flora of mainly woody plants, which represents in modified form the Tertiary flora of Europe. This series of plants, although now appearing to have certain tropical (in one case American) affinities, was not derived from the tropics, but from the Palaearctic region, the case being parallel with that of the snails.

5. We may presume that part of the insular flora has become extinct; thus the dragon-tree (*Dracaena*) has entirely disappeared from Porto Santo, where it was once a conspicuous feature. The fossil plants, however, give as yet no satisfactory evidence.

6. The ease with which plants of many kinds, tropical and subtropical, can be grown in Madeira, indicates that the island is suited to a flora very different from that which actually occupied it. It affords additional support to the view that even in Tertiary times there was no immigration from tropical Africa, or from across the Atlantic. The means of transport were much the same then as now, and apparently there never was a current setting from the south.

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## NOTES ON THE STRUCTURE OF PENNSYLVANIAN PLANTS FROM ILLINOIS. II

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(WITH PLATE VI AND SEVEN FIGURES)

The following notes are based on thin sections of coal balls, collected by Dr. A. C. NOÉ or by the writer from the Pennsylvanian of Illinois, and are presented in three parts.

1. Practically nothing is known concerning the structure of the leaves of *Calamites* known as *Annularia*, although they are not uncommonly found as impressions. Occurring very abundantly in a series of preparations are sections of leaves or bracts which are readily identified as Calamitean. The cross-section shown in fig. 1 is slightly crescent-shaped, and is expanded laterally to form a lamina. On the concave side is a median ridge which marks the position of the single vascular bundle, toward which the edges of the lamina are sometimes slightly pointed.

The cells of the epidermis are elongated toward the extremities of the organ. Those on the convex surface are generally larger and are always covered with a thick cuticle, which in some instances is equal to half the diameter of the epidermal cell. The cuticle is less in amount on the concave surface, although well developed opposite the vascular bundle.

The single median vascular bundle is situated very near the adaxial surface. Surrounding the vascular tissue is the bundle sheath of large cells, often somewhat irregular and occasionally more than one cell in thickness. These cells of the sheath above and below the bundle invariably have that dark content which gave rise to the term "melasmatic layer" (3), but usually this cell content is less in amount or even absent in the cells of the sheath lateral to the vascular bundle.

The bundle itself is composed of a circular group of spirally thickened tracheids (fig. 2), about seven or eight in number and of very small caliber, surrounded by a few irregular rows of thin-walled

elongated cells, slightly larger in diameter, which no doubt include what phloem is present. In longitudinal section it is apparent that the tracheids do not always constitute a solid strand, but are sometimes separated by cells with unthickened walls.

There appears to be no fibrous or mechanical strand as described in certain Calamitean leaves of the *Asterophyllites* type (5). The elongated columnar cells, which are very similar in appearance to the palisade tissue of modern foliage leaves, constitute most of the tissue outside the bundle sheath. They are absent and adaxial to the bundle, however, and are modified laterally to it, where the cells are



FIG. 1. - Transverse section of leaf or bract of *Calamites*;  $\times 45$ .



FIG. 2. - Longitudinal section cutting obliquely through vascular bundle, showing spiral thickenings of tracheids;  $\times 45$ .

of larger diameter and approximately isodiametric. A longitudinal section (fig. 3) passing to one side of the vascular bundle shows the large intercellular spaces separating the parenchyma cells of the lamina.

Positive identification of stomata is lacking; certainly

they were absent on the convex surface. The evidence, however, points very strongly to the occurrence of stomata on the adaxial surface on either side of the midvein.

A longitudinal section parallel with the lamina measures about 1.6 mm. in width. The actual width as seen in transverse section is

about 2.6 mm. A vertical section through the region of the median vein measures about 0.63 mm. It is impossible to give an estimate of the length, as the longitudinal sections of several millimeters gave no indication of either extremity. Nothing is known, therefore, concerning the attachment of this organ to an axis.

RENAULT (4) figured a very similar transverse section which agrees well in size and general features with these specimens, but his illustrations give no details of the bundle. He thought that his section might well be that of a sterile bract of *Bruckmannia grand'euryi*, a *Calamostachys*.



FIG. 3.—Longitudinal section to one side of vascular tissue showing arrangement of parenchyma tissue of lamina;  $\times 45$ .

LADY ISABEL BROWNE (1), in a note without figures, described the sterile bracts of *Palaeostachys gracilis* Ren. Her description in many ways is similar to the one just given. She especially mentions the position of the vascular bundle as being near the adaxial surface. The measurements given in her note are smaller than those given here.

More recently CARPENTIER (2) has described a section which seems to coincide with the description given here in most details. He identified his specimen as the leaf or bract of *Calamites*. There is reason for referring these specimens to the leaves of *Calamites* of the *Annularia* type. A close comparison with the anatomy of the leaves of the *Asterophyllites* type as described by THOMAS shows an extraordinarily close agreement with certain of his types, not only in general appearance, but in most details. The dimensions of the sections

here described compare favorably with the size of impressions of species of *Annularia*, and the numerous transverse sections would seem to indicate an organ with little gradation in width, as the whole series is remarkably uniform in width and thickness. The longitudinal sections show no variation of size or structure. It must be remembered, however, that no indication of attachment was found in either transverse or longitudinal sections, so that the question must continue to remain open.

2. A petrified stem of *Lepidodendron* was collected from the McLeansboro formation (fig. 11). The specimen when found was some 12 cm. long, with a diameter of about 2.5 cm. It was entirely decorticated, with every indication that an unknown amount of secondary wood had been lost before petrification occurred. The preservation is exceptionally good.

The primary wood has a thickness of about twelve cells, which are rather uniform in size, an average cell measuring 0.126 by 0.180 mm., which is slightly larger than an average cell of secondary wood. The primary wood does not now form a continuous cylinder, but is more or less broken into groups which give the appearance superficially of being individual bundles. The stem has been subjected to considerable pressure, as shearing of the secondary wood shows, so that there is little doubt but that the primary wood originally consisted of an unbroken cylinder, which is indicated in some sections by a nearly complete ring.

The pith cavity is entirely destitute of cellular tissue, whether due to loss before petrification or whether normally absent in a stem of this size cannot be determined. Numerous leaf traces arise from the protoxylem on the outer face of the primary wood, and follow a horizontal course through the secondary wood. The rays are usually one cell in width, and frequently contain elements with spiral or scalariform thickenings.

Longitudinal sections show the usual spiral markings of the exarch protoxylem, and the scalariform thickening on both radial and tangential walls of the metaxylem and secondary wood.

*Stigmaria* is not uncommon in the Illinois coal balls. In transverse section (figs. 8, 9) the tracheids are almost entirely radially arranged, with no sharp distinction between primary and secondary

wood. The smallest tracheids with spiral markings are next to the pith cavity.

The woody cylinder is more or less divided into bundles by the primary rays. Secondary rays are numerous and are usually one cell in width. A large proportion of the secondary rays contain elongated elements with spiral or scalariform thickening. With the exception of the protoxylem, all the tracheids have scalariform markings on both radial and tangential walls. The last formed cells of the secondary



FIG. 4.—Transverse section of "rootlet" of *Stigmara ficoides*; all tissues shown.

wood are distinctly smaller than the others, and in many cases are scarcely radial. This has been noticed frequently in British specimens of *Stigmara*.

The tissue immediately surrounding the xylem is not well preserved in these sections. There is the usual periderm formation, exterior to which is the parenchymatous region of the outer cortex.

As is usually true, the stigmarian appendages, commonly called "rootlets," are the most common fossils. In some instances all the tissues of the appendages are well preserved (fig. 4), but in the usual state of petrification the middle cortex is entirely lost, as are also the delicate cells immediately surrounding the xylem of the stele.

The xylem of the stele is in connection with the innermost tracheids of the stigmarian axis, and follows a principal ray outward. A characteristic appearance of an outgoing rootlet trace is shown in the tangential section of *Stigmaria* (fig. 10). The xylem of the "rootlet" is more or less triangular in transverse section with the protoxylem at one apex. The stele is therefore monarch. Many of these stigmarian appendages have developed secondary wood (fig. 5).

It appears that the structure of the axis and "rootlets" of these specimens is that which is described for *Stigmaria ficoides*, recogniz-



FIG. 5.—Transverse sections of stele of a *Stigmaria* appendage showing single protoxylem point and development of secondary wood.

ing the probability that the rather large variation of forms now included in this species may eventually be separated specifically.

3. Isolated *Psaronius* rootlets are commonly found in Illinois petrifications. A small *Psaronius* stem is too disorganized to permit an accurate description, although it is obvious that certain of the isolated rootlets belong to quite another species than that of the stem.

The steles of the rootlets have the usual radial arrangement, with the smallest tracheids with spiral thickenings at the extremities of the arms. The larger tracheids are scalariform. There is an unusually small number of protoxylem points. The larger rootlets are pentarch; the smaller ones are often tetrarch or even triarch. The diameter of the larger rootlets is about 4.6 mm., the stele of which is about 1.2 mm. in diameter.

In some instances the phloem is well preserved, as shown in fig. 6, in which the stele is tetrarch, with the well preserved phloem alternating with the four arms of the xylem and surrounded by the endodermis. The striking lacunar cortex is often preserved with great exactness. Fig. 7 shows a portion of the cortex. Exterior to the

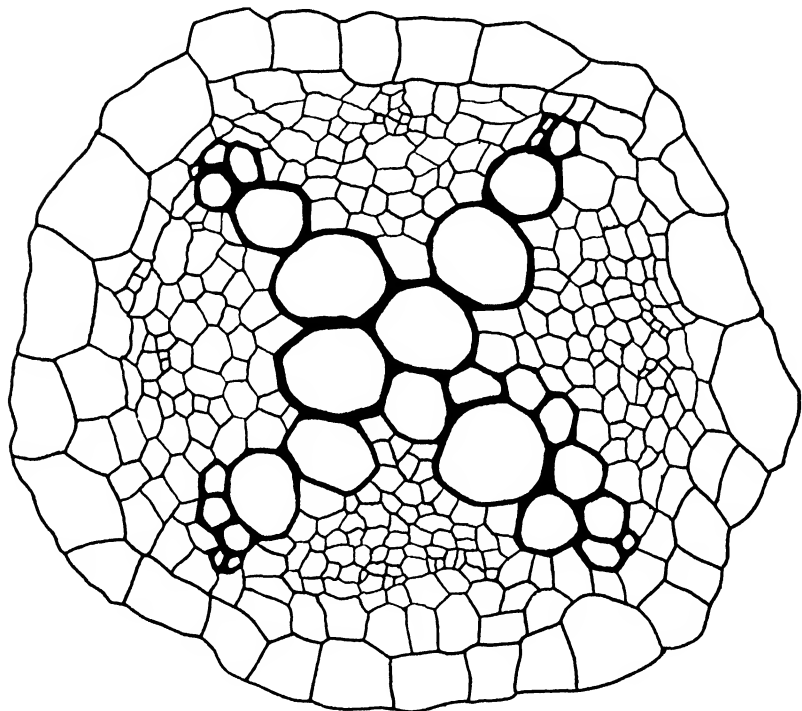


FIG. 6.—Camera lucida drawing of stele of *Psaronius* rootlet showing tetrarch stele;  $\times 135$ .

lacunar tissue is a narrow zone of heavy-walled fibrous cells, two or three cells in width. Exterior to this mechanical tissue is a zone of parenchymatous tissue bounded in some instances by an epidermis. In most of the rootlets both the lacunar inner cortex and the parenchymatous outer zone were more or less destroyed before petrifaction.

No hairs were found attached to the larger rootlets with the epidermis preserved. These were probably free outer rootlets. In the

smaller ones the parenchymatous layer external to the mechanical tissue is lacking, a condition usually true of the inner root zone of *Psaronius*, where the sclerenchyma layer is the limiting tissue except

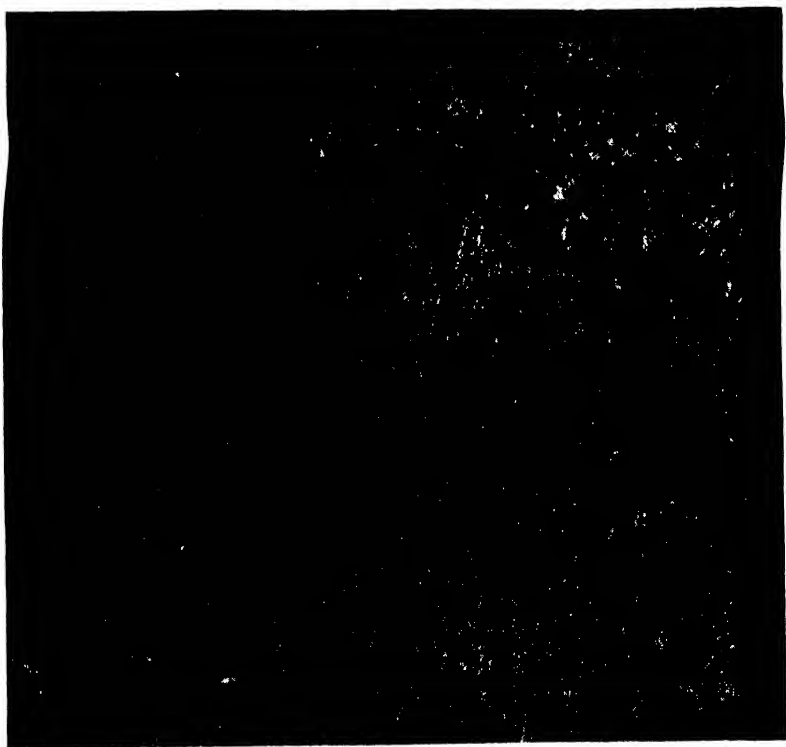


FIG. 7.—Transverse section of *Psaronius* rootlet showing lacunar cortex, narrow zone of mechanical tissue, and outer parenchymatous zone;  $\times 55$ .

where the cells have elongated to form the filamentous interstitial tissue.

This investigation was initiated while the writer was a National Research Fellow in the Biological Sciences, and he wishes to express his great appreciation of the opportunity afforded by the financial assistance of the National Research Council.

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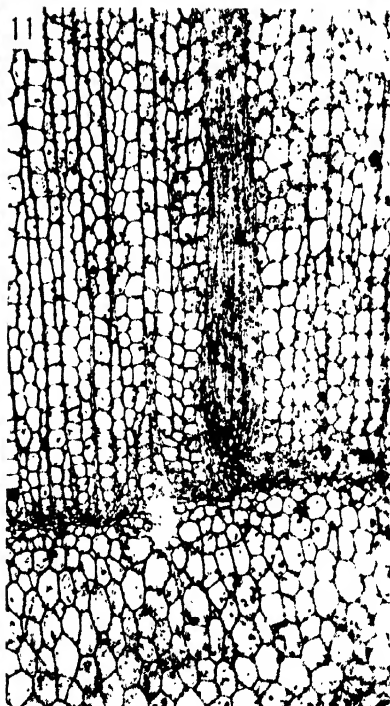
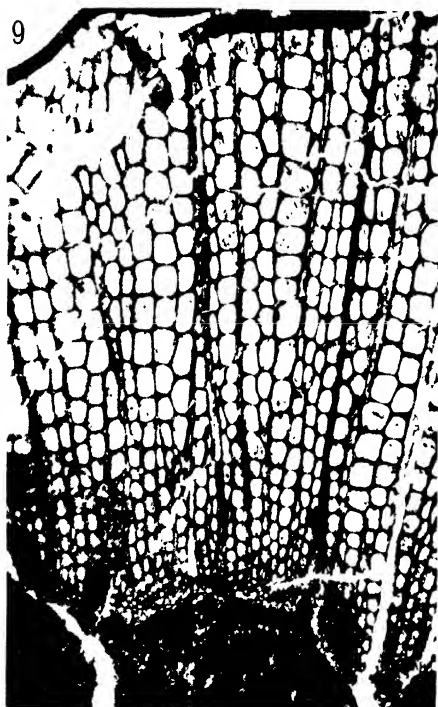
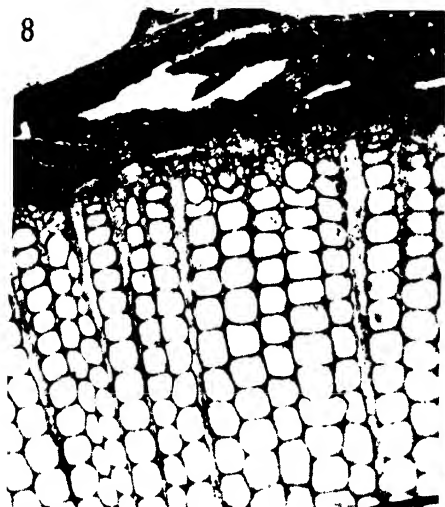
## EXPLANATION OF PLATE VI

FIG. 8.—Transverse section of outer portion of stele of *Stigmaria ficoides*: latest formed tracheids are smaller and scarcely radial;  $\times 32$ .

FIG. 9.—Tangential section of stele of *S. ficoides* showing an outgoing "rootlet" trace;  $\times 16$ .

FIG. 10.—Transverse section of inner portion of stele of *S. ficoides*;  $\times 27$ .

FIG. 11.—Transverse section of portion of stem of *Lepidodendron* showing primary and secondary wood;  $\times 30$ .





# NORTHERN ASPECT AND PHENOLOGY OF TALLAHASSEE RED HILLS FLORA

HERMAN KURZ

(WITH TWO FIGURES)

## Northern disjuncts

To the average northern botanist, Florida is a peninsula projecting southward from the southeastern United States, where sand, swamps, lakes, sunshine, everblooming and tropical or subtropical plants are the rule. Few realize that topographically and geologically middle northern Florida is but an extension of Alabama and Georgia. A generalized map (fig. 1) after HARPER (1)<sup>1</sup> shows the most important features of these geographic regions in the form of a taxonomic key. A portion follows:

Soil largely red clay; topography rather hilly

Streams common

Limestone outcrops frequent; caves and natural bridges

present.....Marianna Red Lands

Limestone impure, cropping out only on bluffs; no caves

Apalachicola River bluffs

No rock outcrops.....Knox Hill Country

Streams scarce

No lakes.....Holmes Valley

Lakes and ponds common.....Tallahassee Red Hills

The Knox Hill Country and Holmes Valley will not be discussed here. The most marked features of the Marianna Red Lands, the Apalachicola River Bluffs, and the Tallahassee Red Hills are the relatively rugged red clay hills. Their altitude is usually less than 300 feet above sea level, but they are frequently steep. The relatively undisturbed places contain mesophytic climax forests of magnolia, beech, maple, sweet gum, elm, ash, oak, hickory, basswood, tulip tree, hackberry, black cherry, mulberry, spruce pine,

<sup>1</sup> See the same paper for the geology, topography, soil, vegetation, and citations to older work.

and others. Several species of pines and oaks dominate the areas in the process of reforestation. These hilly regions are surrounded either by gently rolling dry, sterile, sand hills with long leaf pine and species of scrub oaks, or by waterlogged flatwoods, with long

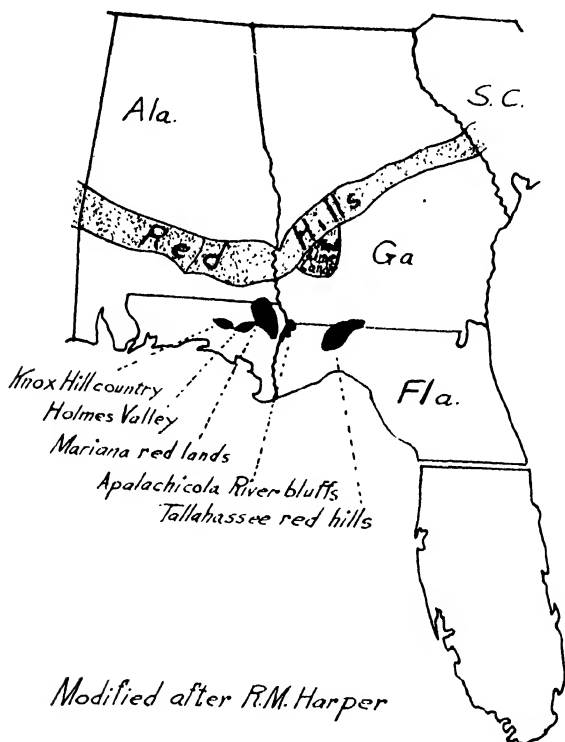


FIG. 1

leaf pine, wire grass, and various ericads, sarracenias, orchids, and so forth. This monotony is often broken by lakes, ponds, or gum, cypress, and bay swamps. Essentially, then, the three regions in question may be considered as hilly "islands" of rich loam or humus soils dominated by hardwood or pine-oak forests, and surrounded by an "ocean" of rolling sandy country or water-logged flatwoods.

That a number of northern herbs reach their southernmost limits in the rich and cool forests of the Marianna Red Lands and Apalachicola River Bluffs is well known. Naturally their range is there-

fore extended to Florida in SMALL's *Flora of the Southeastern United States* and other books, but it should be emphasized that up to the present time these plants as a class have been known to occur only in the two very local areas of northern Florida just mentioned, where they appear again as northern disjuncts, after having skipped the scrub oak-pine lands and flatwoods of southern Alabama and Georgia.

The Tallahassee Red Hills, with their cool, rich mesophytic forests, form a third island suitable for a number of hitherto unreported northern disjuncts. A list follows:

Arisaema triphyllum <sup>2</sup>	Polygala polygama
Muricauda draconium	Sanicula marylandica
Uvularia perfoliata	Spigelia marylandica
Oakesiella floridana	Phlox pilosa (probably)
Salomonina biflora	Penstemon hirsutus
Trillium underwoodii	Pedicularis canadensis
Corallorhiza wisteriana	Hexastylis callifolia
Ranunculus palmatus	Senecio aureus
Thalictrum purpurascens	Senecio obovatus
Sanguinaria canadensis	

Most of these are found northward as far as New England and Canada. A few, like *Trillium underwoodii*, *Phlox pilosa*, *Oakesiella floridana*, and *Hexastylis callifolia*, suggest the north by virtue of their more northern close relatives. Besides these, *Monotropa uniflora*, *Epifagus virginiana*, *Conopholis americana*, and *Antennaria plantaginifolia*, also reported by Miss GANO (2), might be mentioned.

BRADFORD TORREY (3) states that "ground flowers were very little in evidence" around Tallahassee in spring. As most of the species are rare, naturally he missed them. While some habitats nearby do not belong to the Red Hills proper, they contain an additional number of northern or semi-northern ground flowers. Notable ones are *Lupinus nuttallii* (not very different from *L. perennis*), *Amsonia ciliata*, and *Phlox floridana* of the xerophytic sterile sands; *Sarracenia purpurea*, *Limodorum tuberosum*, *Pogonia ophioglossoides*,

<sup>2</sup> All species were classified according to SMALL's *Flora of the Southeastern United States*.

*P. divaricata*, *Ibidium cernuum*, *Atamasco atamasco*, *Viola lanceolata*, and *V. primulifolia*; *Kalmia latifolia* along certain streams; and *Iris carolina* and *Amsonia amsonia* in certain marshes. This group, while not so restricted as the list given for the Red Hills, nevertheless serves to accentuate the northern aspect of the region as a whole. The northern aspect is still further reinforced by the data of the following phenological study. Out of a total of 319 flowering plants, naturals<sup>3</sup> and ruderals,<sup>4</sup> observed, about three-fourths extend northward at least to Virginia; and of these about one-half reach as far as Minnesota, Illinois, Ohio, New Jersey, New York, New England, and northeastern Canada. Finally, to offset those northern flowers actually missing, we find, especially in the "scrub oaks" and flatwoods, southern species of lupines, orchids, pinguiculas, sarracenias, sundews, composites, and others.

### Phenology

Nothing definite has appeared about the spring flowering season of the Tallahassee vicinity. Miss GANO does say that "There is no vernal flora, nor can a definite flowering season be set, but there is overlapping and irregularity in the prolongation of the blooming season, conditions related to the spring drought and to the extended growing season due to climatic causes." This statement does not fit the facts exactly, however, for the leafless deciduous trees and shrubs and the many dead autumn herbs in chilly January suggest at once a lull in vegetational activity, which, while not so extreme, is yet comparable with the winter conditions of the north.

With the purpose of acquiring some information about the "vernal flora," "overlap," "irregularity," and "prolongation of growing season," therefore, blooming dates for 319 species growing within 25 miles of Tallahassee were taken. Such a radius of course takes in a variety of habitats not typical of the Red Hills. Beginning with January 1 and ending May 15, 1925, one or more trips a week were made into the field in order to catch the earliest or nearly earliest blooming dates. The total number of new naturals and ruderals

<sup>3</sup> A word adapted by the writer for wild plants characteristic of relatively undisturbed areas.

<sup>4</sup> Plants, native and introduced, of old fields, waysides, etc.

are plotted separately on the graph, each species being counted only once. It is now realized that duration of blooming periods of a representative number of species should have been recorded. However, since the dates have at least been partially checked, it is felt that even this preliminary work can be relied upon for a general idea of the seasonal periodicity. Mr. W. H. MARKHAM, the local United

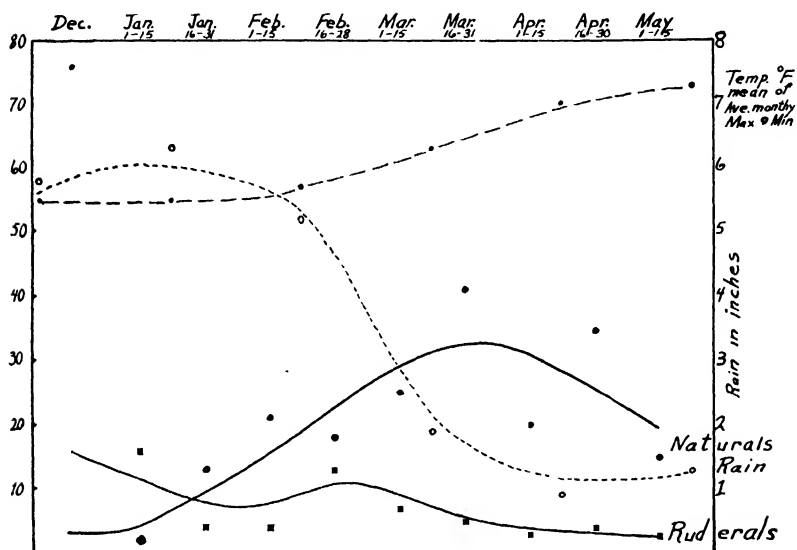


FIG. 2

States Weather Bureau observer, furnished the temperature and rainfall data. Additional meteorological data were not available (fig. 2).

In the autumn of 1925 a number of trips were made in order to ascertain approximately the duration of the autumn bloomers. The following was found:

OCTOBER AND EARLY NOVEMBER		DECEMBER	
Naturals.....	31	.....	4
Ruderals.....	21	.....	11

Among the few naturals continuing into December are *Hamamelis virginiana*, *Dasystephana tenuifolia*, and *Aster* spp. *Monotropa uniflora* blooms in October and early November, whereas its time for Long



Island and Illinois is in July and August. At St. Petersburg it is reported to bloom at Christmas, so that this is an example of one of the summer bloomers of the north that flower progressively later southward rather than earlier, as do most of the typical spring bloomers common to both north Florida and the northern states. Examples of autumn ruderals lagging occasionally over into December and even January are *Solanum nigrum*, *Onagra biennis*, *Ambrosia artemisiifolia*, *Helenium tenuifolia*, *Sida acuta*, *S. rhombifolia*, *Malvastrum americanum*, *Nothoscordum bivalve*, *Oxalis stricta*, etc., the last two continuing more or less throughout the winter. Most important, however, in dovetailing late fall with early spring<sup>s</sup> flowering are such winter annuals or biennials as *Sitilias caroliniana*, *Gnaphalium purpureum*, *Sonchus asper*, *Argemone mexicana*, *Erigeron philadelphicus*, *Lamium amplexicaule*, *Veronica arvensis*, *V. agrestis*, *V. peregrina*, *Houstonia minor*, *Alsine media*, *Draba brachycarpa*, *Coronopus didymus*, and others. About the beginning of February these are replaced or supplemented by such species as *Linaria canadensis*, *Medicago arabica*, *Specularia perfoliata*, *Plantago virginica*, *Spermo-lepis divaricatus*, *Chaerophyllum tainturieri*, and others.

These lists, while not by any means exhaustive, will suffice to demonstrate the part these common ruderals play in the overlap of flowering. While they are hardy as a class, their activity may nevertheless be checked or delayed temporarily.

To them, also, may be attributed most of the irregularity and everblooming spoken of by Miss GANO. Excepting *Polygala lutea*, *P. nana*, and a few asters, no naturals have been noticed blooming all winter, so that everblooming by this class is negligible. In January the first naturals appear. From then on they flower in increasing numbers until some time in March, after which there is a gradual decline. They exhibit therefore a periodicity which, while not so abrupt (although earlier than in the north), is yet rather definite. As expected, even the blooming of naturals may be delayed during relatively severe winters. During the winter of 1925-26, when the temperature averaged about 6° F. lower than in 1924-25, the class as a whole flowered about two weeks later, but there was no irregularity.

<sup>s</sup> Spring in terms of flowering activity of naturals begins here in January.

Whether or not the Tallahassee vicinity can boast of a vernal flora depends on the rigidity of definition. However, the foregoing data ought to prove that there is a vernal flora, at least in spirit, if not in letter.

The writer owes much to Dr. R. M. HARPER for his suggestions and criticisms, and for his aid in the location of a number of stations for rare species and their identification. Thanks are also due to Dr. RAYMOND BELLAMY for aid in field observation. .

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SOME NEW AND LITTLE KNOWN SPECIES  
OF TRACHELOMONES FROM NORTH  
MANCHURIA, CHINA

B. W. SKVORTZOW

(WITH PLATE VII)

The summer of 1924 was spent by the writer at the biological station of the Manchuria Research Society at Harbin, who during that time studied the algae of the district surrounding the station. This paper presents a list of the new forms of *Trachelomonas* EHRENBERG discovered in marshes, ponds, and lakes of the Sungary River valley, and also descriptions of some little known species from Europe and other countries.

*TRACHELOMONES TUBEROSA* Skv., Jour. Asiatic Soc. Shanghai 4:53. 1919; PASCHER, Archiv Protistenkunde 48:504. 1924.—Shell elongated, variable in shape, contracted at both ends, brown, 28 by 13  $\mu$ ; neck 4.5  $\mu$  broad; chromatophores numerous; stigma distinct; cilia 1.5–2 times longer than shell.—Fig. 1.

In marshes near Harbin.

*TRACHELOMONES TUBEROSA conspersa*, var. nov.—Shell brown and covered with knobs, 33.3 by 18.5  $\mu$ ; chromatophores 8; stigma distinct; cilia 1.5 times longer than shell.—Fig. 2.

In marshes near Harbin.

*Trachelomonas cucurbita*, sp. nov.; *T. helvetica* Lemm. var. *hispidula* Skv., Jour. Asiatic Soc. Shanghai 4:72. 1919.—Shell brown, covered with sharp pointed spines, elongated, contracted at both ends and rounded at the end, 30–35 by 14.5–15.7  $\mu$ ; cilia aperture 4.2  $\mu$  in breadth; chromatophores numerous.—Fig. 3.

In marshes near Harbin.

*TRACHELOMONES CUCURBITA ovata*, var. nov.—Shell brown, smooth, 25–27 by 10.2–12  $\mu$ , tubelike neck 4.4 by 3.4  $\mu$ ; in other respects similar to the species.—Fig. 4.

In marshes near Harbin.

*Trachelomonas vestita*, sp. nov.; *T. hexangulata* Swir. var. *sinica* Skv., Jour. Asiatic Soc. Shanghai 4:193. 1919.—Shell oblong or

hexagonal, with rounded end, 35  $\mu$  long, brown, lower part covered with spines, tubelike neck serrated.—Fig. 5.

Known only from marshes in South China.

**Trachelomonas schewiakoffii**, sp. nov.; *T. rhombica* Skv. var. *planktonica* Skv., Jour. Asiatic Soc. Shanghai 4:53. 1919.—Shell oval, contracted at both ends, brown, smooth, 30 by 20  $\mu$ ; neck 5.5 by 3.5  $\mu$ ; chromatophores numerous.—Fig. 6.

Named in honor of Dr. W. T. SCHEWIAKOFF, well known Protistologist of Irkutsk University, Siberia, Russia.

TRACHELOMONES SCHEWIAKOFFII var. **polonica** (Koczw.) nob., comb. nov.; *T. polonica* Koczwara, Kosmos, Lwow 40:231-275. 1915.—Shell oval, contracted at both ends, more pointed at end, 35-38 by 15-17  $\mu$ , tubelike neck 4  $\mu$  long.—Fig. 7.

Known only in Biatogorski Lake in Polen.

**Trachelomonas wolozynskii**, sp. nov.; *T. curystoma acula* Lemm. Kryptog. Mark Brand. 3:528. 1910; STEIN, Ker Organismus der Infusionstiere 3:1878; PASCHER et LEMMERMAN, Die Süßwasser Flora Deutschlands 2:151. 1913.—Shell brown, smooth, rhomboidal with rounded end and very broad middle part, neck cut obliquely; chromatophores numerous.—Fig. 8.

Known from Europe.

TRACHELOMONES WOLOZYNSKII ASPERA Skv.; *T. regularis* (Lemm.) Skv. *asperum* Skv. Jour. Microbiol. 4:71. 1917, and Winter phytoplankton of the fish ponds of Foochow 53:193. 1922.—Shell more elongate, 27.8 by 13  $\mu$ , brown, covered with knobs, neck straight; cilia 1.5 times longer than shell; chromatophores numerous.—Fig. 9.

Known from South China and Manchuria.

TRACHELOMONES WOLOZYNSKII **longicollis**, var. nov.; *T. regularis asperum* Skv. *forma*, Winter phytoplankton of the fish ponds of Foochow 53:193. 1922.—Shell brown, covered with knobs, contracted at both ends and broadly rounded, 27 by 12  $\mu$ , upper part passing directly into serrated neck.—Fig. 10.

Known only in South China in fish ponds.

**Trachelomonas kozlovii**, sp. nov. —Shell oval, rough, transparent, 81.4 by 47  $\mu$ , upper part contracted and passing directly into neck cut obliquely and 7.4  $\mu$  broad, lower part contracted and little

drawn out and rounded; chromatophores numerous; stigma distinct; paramylons small and round.—Fig. 11.

Found only near Harbin.

Named in honor of I. W. Kozlov, who collected this form.

**Trachelomones rapacea**, sp. nov.; *T. volgensis chinensis* Skv. Jour. Asiatic Soc. Shanghai 4:7. 1919.—Shell brown, covered with knobs, spherical, contracted and pointed at the end, 38–40 by 21–25  $\mu$ ; chromatophores numerous; stigma distinct; paramylons small and round.—Fig. 12.

Known only from South China.

**Trachelomones stagnalis**, sp. nov.; *T. fluviatilis curta* Skv. Jour. Asiatic Soc. Shanghai 4:54. 1919.—Shell brown, broad, oval, contracted at both ends, upper part passing directly into neck 2.5  $\mu$  broad, lower part pointed, 22 by 16  $\mu$ ; chromatophores numerous.—Fig. 13.

In marshes near Harbin.

**TRACHELOMONES TAMBOWIKA SWIRENKO amphora**, var. nov.—Shell oval, smooth, neck enlarged at end to 7  $\mu$  broad, 44.4 by 29.6  $\mu$ , lower part of shell pointed, end spine 11.1  $\mu$  long; chromatophores numerous; stigma distinct.—Fig. 14.

In plankton near Harbin.

**TRACHELOMONES URCEOLATA STOKES** var. **HYALINA** (Swir.) nob.; *T. hyalina* Swirenko, Trav. Inst. Bot. Univ. Kharkoff, no. 26. p. 25. 1915.—Shell transparent, covered with small knobs, elongate oval 62 by 32  $\mu$ , upper part passing directly into serrated neck, lower part pointed; chromatophores numerous, 6.5  $\mu$  in diameter; stigma distinct, 4.5  $\mu$  in diameter; paramylons small, elongate.—Fig. 15.

Known only from ponds in Russia.

**TRACHELOMONES ENSIFERA DADAY** var. **LONGICAUDA** (Swir.) nob.; *T. longicauda* Swirenko, Beitr. Kent. Flagell. der Charkow. 1913.—Shell brown, rough, spherical, contracted and pointed at end, 44–5 by 22–29  $\mu$ , end spine 21  $\mu$  long, upper part passing into neck 12 by 7  $\mu$ , oblique; stigma round, 3  $\mu$  in diameter; paramylons oblong or round.—Fig. 16.

At present found only in ponds of Russia.

**TRACHELOMONES SCHAUNINSLANDII manschurica**, var. nov.—Shell stiff, oval, covered with small knobs, 66.6–70.3 by 30–40.7  $\mu$ ,

end contracted and pointed, upper part passing into oblique neck 5.7–6  $\mu$  broad; chromatophores numerous; stigma distinct.—Fig. 17.

In marshes in North Manchuria.

**TRACHELOMONES INFLATA crenulato-collis**, var. nov.—Shell brown, covered with dots, elongate, oval, contracted at both ends, 66.6 by 31  $\mu$ , lower contracted part rounded and with a small spine at end; neck straight, brown, smooth and serrate, 7.4 by 5.7  $\mu$ , distinct from shell.—Fig. 18.

Known only from plankton near Harbin.

**Trachelomones dangeardii**, sp. nov.; *T. fluviatilis* Lemm. in SWIRENKO, Archiv Hydrobiol. Plank 9:640–645. 1914.—Shell oval, brown, smooth or rough, 25.9 by 11.1–17  $\mu$ , upper part passing directly into neck which is enlarged at end to 5.5  $\mu$  in diameter; chromatophores 4 or 5, without pyrenoids; cilia 2–3 times longer than shell; paramylons small, round.—Fig. 19.

Known only in Russia and North Manchuria.

Named in honor of Professor P. A. DANGEARD, of France.

**TRACHELOMONES DANGEARDII** var. **glabra**, comb. nov.; *T. fluviatilis glabra* Skv. Jour. Asiatic Soc. Shanghai 4:54. 1919.—Shell oval, brown, covered with knobs, 26 by 14.1  $\mu$ , neck oblique; chromatophores numerous.—Fig. 20.

In marshes near Harbin.

**TRACHELOMONES DANGEARDII** var. **LAVERTA** (Swi.) nob.; *T. fluviatilis lacerta* Swirenko, Trav. Inst. Bot. Univ. Karkoff, no. 26, p. 30. 1915.—Shell brown, smooth, spherical, contracted and pointed at end, 32–34 by 14–16  $\mu$ , end spine 10  $\mu$  long, upper part passing directly into serrated neck 7  $\mu$  long and broad.—Fig. 21.

In marshes of Russia.

**TRACHELOMONES HELVETICA manschurica**, var. nov.—Shell brown, covered with sharp pointed spines, oval, contracted at both ends, 40.7 by 18.5  $\mu$ ; neck straight, 5.7  $\mu$  broad, distinct from shell; lower part pointed and rounded at end; chromatophores numerous.—Fig. 22.

In plankton near Harbin.

**TRACHELOMONES SWIRENKO** Skv. **sinensis**, var. nov.—Shell brown dotted and only in lower part covered with knobs, contracted

at both ends, 40.7 by 18.5  $\mu$ , upper part passing directly into neck, 4 by 4.5  $\mu$ ; chromatophores numerous.—Fig. 23.

As yet found only near Harbin.

*TRACHELOMONES FLUVIATILIS* LEMM. var. *LEVIS* (Lemm.), comb. nov.; *T. affinis levis* Lemm., FORSCH. Biol. Sta. Plön 2:157; Kryptog. Mark Brand. 3:530. 1910; PASCHER et LEMM., Die Süßwasser Flora Deutschlands, 2:153. 1913; SKVORTZOW, Winter phytoplankton of the fish ponds of Foochow 43:193. 1922.—Shell oval, contracted at both ends, 60–68.5 by 26–27  $\mu$ , upper part passing directly into neck, which is 5.7 by 5.5  $\mu$ , lower part pointed.—Fig. 24.

Found in Europe and South China.

*Trachelomones maxima*, sp. nov.—Shell oval, contracted at both ends, 103.6–107 by 40.7–44.4  $\mu$ , upper part passing directly into neck, which is 7.4  $\mu$  broad and oblique, lower part pointed; chromatophores numerous; stigma distinct; paramylons round or long.—Fig. 25.

In plankton near Harbin.

*Trachelomones nadsonii*, sp. nov.—Shell brown, contracted at both ends, 66.6 by 22.5  $\mu$ , upper part passing directly into neck, which is 9.2 by 5  $\mu$  and serrate, lower part pointed, end spine 14.8  $\mu$  long; chromatophores numerous; paramylons round.—Fig. 26.

In plankton near Harbin.

Named in honor of Dr. G. A. NADSON of Leningrad, Russia.

*Trachelomones baikovii*, sp. nov.—Shell brown, covered with spines, oval, contracted at both ends, 51.8–55.5 by 23–25.9  $\mu$ ; neck straight, serrate, 7.4 by 5.7  $\mu$ , distinct from shell; lower part of shell with thick rounded spine; chromatophores numerous; stigma distinct; paramylons small; cilia larger than shell.—Figs. 27, 28.

Found near Harbin.

Named in honor of N. A. BAIKOV, a well known naturalist in Manchuria, who has found this form.

*TRACHELOMONES ACUMINATA* (Schmarda) Stein; *Lagenella acuminata* Schmarda, Denkschr. Wiener Akad. Wiss. 1:1850; LEMMERMANN, Kryptog. Mark Brand. 3:525. 1910; PASCHER et LEMMERMANN, Die Süßwasser Flora Deutschlands, 2:151. 1913; SKVORTZOW, Winter phytoplankton of fish ponds of Foochow, p. 192.

1922.—Shell smooth, trapezoid, 50–59  $\mu$  long, upper part passing directly into oblique neck, lower part pointed; cilia twice as long as shell.

Known from Europe and South China.

TRACHELOMONES ACUMINATA *triangulata*, var. nov.; STEIN Der Organismus der Infusionstiere 3:1878; LEMMERMANN, Kryptog. Mark Brand. 3:151. 1910; PASCHER et LEMMERMANN, Die Süßwasser Flora Deutschlands, 2:151. 1913.—Shell triangular; in other respects similar to typical form.—Fig. 29.

Known from Europe.

TRACHELOMONES ACUMINATA VERRUCOSA Teodoresco, Beih. Bot. Centralbl. 21:215; SKVORTZOW, on phytoplankton from ponds of Tientsin, p. 189. 1922.—Shell brown, covered with knobs, 26–34 by 14–22  $\mu$ .—Fig. 30.

Found in Rumania and North China.

TRACHELOMONES ACUMINATA MAJOR Skv., Jour. Microbiol. 4:70. 1917.—Shell transparent, smooth, 90–92.9 by 37.4–38  $\mu$ , neck oblique; chromatophores numerous; stigma distinct.

In plankton from Manchuria.

HARBIN, MANCHURIA  
CHINA

#### EXPLANATION OF PLATE VII

FIG. 1.—*Trachelomonas tuberosa*.

FIG. 2.—*T. tuberosa conspersa*.

FIG. 3.—*T. cucurbita*.

FIG. 4.—*T. cucurbita ovata*.

FIG. 5.—*T. vestita*.

FIG. 6.—*T. schewiakoffii*.

FIG. 7.—*T. schewiakoffii polonica*.

FIG. 8.—*T. woloszynskii*.

FIG. 9.—*T. woloszynskii aspera*.

FIG. 10.—*T. woloszynskii longicollis*.

FIG. 11.—*T. kozlovii*.

FIG. 12.—*T. rapacea*.

FIG. 13.—*T. curta*.

FIG. 14.—*T. tambowika amphora*.

FIG. 15.—*T. urceolata hyalina*.

FIG. 16.—*T. ensifera longicauda*.





# VASCULARIZATION OF THE NODE IN ZEA MAYS

ARTHUR T. EVANS

(WITH SEVEN FIGURES)

The fate of the vascular bundle, once it reaches the node of the stalk in a corn plant, has remained a baffling situation for years. Imbedding of tissue from the node and then sectioning has been found inadequate in the study of the node, since the branching of the vascular bundle is so intricate and the branches so tortuous as to defy solution, even in the best prepared serial sections. Single vascular bundles passing through the node may clearly be demonstrated by sections, but they do not definitely represent the true situation.

The writer has been interested in a study of the vascular system in the corn plant for a number of years. It was not until recently, however, that a technique capable of showing the true situation in the node was developed.

## Material

A number of young corn plants were collected and an effort made to trace the vascular system by staining it with methylene blue. When the whole end of the stalk was inserted into the stain, it was found to become so completely colored as to obscure the course of individual bundles through the nodes. Later, a number of stalks were cut away in such a manner as to leave only three or four bundles, about an inch long, protruding from the end. These bundles, which were in the center of the stalk, were inserted into a container of methylene blue so that they alone were in a position to conduct the stain. This method was found to be unsatisfactory, however, since the plant soon wilted and died. The method was later improved, the few strands to be stained being inserted into a small vial of methylene blue and the mouth of the vial thrust up into the base of the stalk. The end of the stalk could then be placed, bottle and all, into water and the plant thus kept alive (fig. 1).

Through the use of this method the methylene blue was taken up readily by the bundles before the plant wilted. The right degree of

staining could be obtained by proper manipulation. The bundles could then be clearly traced through several nodes of the plant. Any

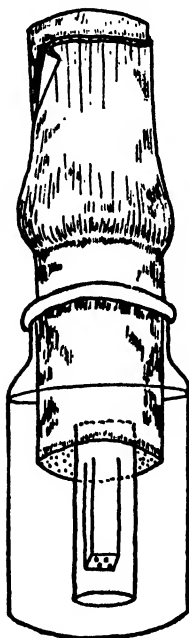


FIG. 1.—Apparatus for staining a few vascular bundles at a time. Stalk is cut as indicated, leaving only three or four vascular bundles protruding; small vial filled nearly full of methylene blue is placed over these and mouth inserted into base of corn stalk; end of stalk is then placed in bottle of water, upper part being left intact. In this manner stalk remains alive for many days.

branching that occurred was easily noticed in the nodes. At first it was decided to dissect out the stained bundles, but this became a hopeless task; although easy in the internodes, it became far too intricate in the nodes. Some of the stained material was placed in a warm place for a few days, and, when examined later, it was found that it had been retted by the action of bacteria, so that the parenchymatous cells were easily removed by washing. This left the stained nodal bundles as a network of vascular strands, which showed excellently under the binocular microscope. These vascular strands in freshly killed material are so elastic that they will withstand a great amount of rough usage.

After washing away thoroughly all the parenchyma with its protoplasm, the specimens were placed in alcohol and later in glycerine, where they were readily kept and cleared into beautiful specimens, the blue bundles showing up well when freed from the obscuring tissues which surrounded them.

ARTSCHWAGER,<sup>1</sup> in a paper dealing with the anatomy of the sugar cane, discusses the vascular situation in the nodes. He states that many of the bundles pass through seven or eight nodes, but that their

absolute length cannot be determined with certainty. STRASBURGER<sup>2</sup> states that in *Zea mays* the longest leaf trace passes through six

<sup>1</sup> ARTSCHWAGER, ERNST, Anatomy of the vegetative organs of sugar cane. Jour. Agric. Res. 30:197-221. 1925.

<sup>2</sup> STRASBURGER, E., Über den Bau und die Vorrichtungen der Leitungsbahnen in den Pflanzen. Histologische Beiträge. Heft III. pp. 1000, illustrated. Jena. 1891.

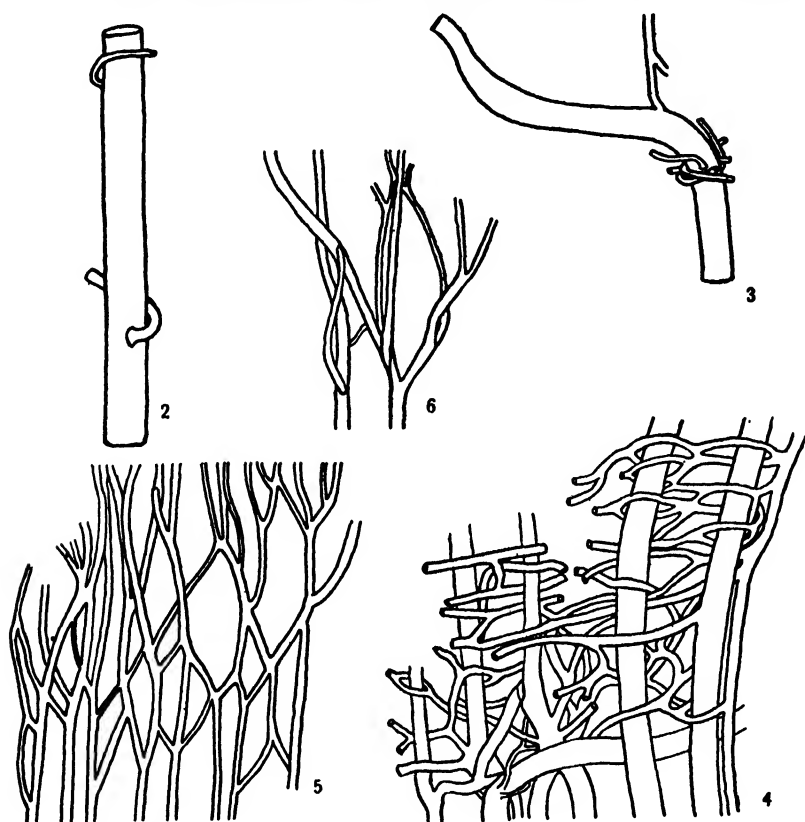
internodes. In this investigation no bundle was found to pass through more than three nodes without branching. There can be no difficulty in noting this, as each bundle may be dissected out in its entirety. There are few bundles which do not give off at least a small branch at each node. Often these small branches are so minute as to escape unnoticed unless examination is made with some magnification. In some cases there appear to be no large branches given off for several nodes. The writer is of the opinion that there is a considerable amount of variation in this matter. In many cases the bundles branch more or less at each node; again, they may not.

The branching of the bundles usually occurs as the bundle enters the node. In this position it is enlarged, and it is from such an enlargement that the branches arise. There are few, if any, of the principal vascular strands which pass through a node without at least some slight alteration. Many are merely deflected to one side or the other; others are flattened and widened; and again they develop the small branches mentioned previously. It can be said with certainty that only a very small number pass uninterrupted through the node; the great majority either branch, change their course, or are modified in form. To be sure, a great number of the bundles pass directly through the node with little more than very slight modification. Such bundles may traverse through one or two nodes before entering the leaves or branching profusely; in some cases the entire bundle is dissipated into a mass of small branches. Fig. 2 represents a single bundle which gives rise to but two small branches into the network of node bundles. This bundle, before dissecting out, appears to pass through the node without branching.

In fig. 3, which is a drawing of one of the large central bundles, showing its course out toward the epidermis to enter the leaf, a great number of branches are given off at the point where it enters the node. A study of the node shows that a large mass of the intertwining bundles which make up the node complex arises at this level, which is just at the point of entry of the bundles.

Fig. 4 is a portion of a section from a node, showing the intricate manner in which the bundles interlace. At the extreme right may be seen a small bundle that gives rise to a series of nodal branches which loop about the nearby vascular strands. It is such small periph-

eral bundles as these that are largely responsible for the network of bundles which give rise to the network of fibers found in the upper



FIGS. 2-6.—Fig. 2, single vascular bundle showing two small branches arising at different levels; fig. 3, single vascular bundle showing course as it passes from center of stalk into leaf, small branch continuing upward through node; number of small branches arise from bundle at point near lowest part of node; bundle flattened and somewhat widened; fig. 4, detailed drawing showing course of various small nodal bundles; note looping of small branches about vascular bundles in upper part of node; fig. 5, number of bundles as seen in outer part of stalk, showing anastomosing character; fig. 6, intricate method of branching found in node.

part of the nodes. These join with the branches which arise from the larger vascular bundles at lower levels to form the complicated vascular situation of the node. Fig. 5 represents a number of these peripheral bundles as they lie just beneath the cortex. This drawing

is a view of the meshwork of peripheral bundles such as are found in the vicinity of each node. Although the smaller peripheral bundles appear to be responsible for most of the minute branches in the node complex, there is a comparable number of these smaller branches which arise from the larger vascular strands as they enter the node. These small branches continue to divide and subdivide through the node, and eventually anastomose with other small branches from other bundles, thus finally becoming joined with other vascular elements at the same level or slightly above the point of their departure from the original bundle. In no case is there a general attachment of the numerous small fibers with the main vascular bundles at high levels in the node. Although an occasional branch unites at some distance above the lower edge of the node, this is not the general situation, since a preponderance of the bundles branching in any node do so from the definite enlargement near the lower edge of the node proper. From this point they ramify through the parenchymatous tissue of the node in all directions, many becoming united with similar small branches from the peripheral bundles. So complicated does the branching become that it is impossible of tracing, even when no other tissues are present to complicate the situation. Many of these small branches subdivide and fuse with others, until they are completely lost in a maze so intricate as to become bewildering (fig. 4).

The methylene blue conducted through the vascular bundles, once it reaches the node, spreads confusingly through it, showing that there is no fixed path by which materials traverse the structure. Apparently materials of whatever type, once they reach the node, pass through any bundle into which chance may carry them. The bulk of the stain remains in the main vascular bundle, and passes directly through the node into the leaf, or into the many branches which arise from it, as the case may be. When the stain enters the small secondary branches of the node, it may often be carried some distance across it, and even into vascular bundles which are without stain when they enter the node.

Fig. 7 is a photomicrograph of a section of the retted node, and shows well the network of bundles. It will be noted that one of the large bundles gives rise to several small branches at its lowest level

in the node. Other branches may be seen to pass off at higher levels. The figure shows the branches passing from higher to lower levels in the node, as well as back and forth across it. Also the main bundle (*B*) shows how such a structure traverses through the node after giving off branches. Two small branches are seen to arise from this

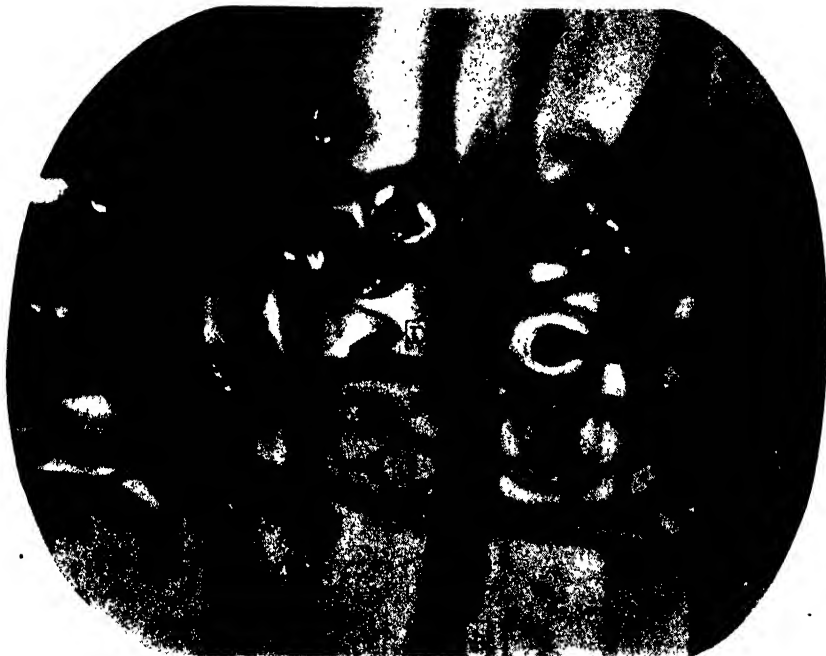


FIG. 7.—Photomicrograph of portion of retted node, showing passing of many small branches in every direction: vascular bundle *A* bends toward leaf after branching; *B* passes more or less directly through but gives off branches at two levels; *C* and *D* show passing of small branches from one level to another and their method of uniting. Photomicrograph by LEITZ.

bundle at its lowest point; one is attached on the upper left surface, the other on the lower right. At *C* one of the numerous small bundles to be found in every node connects the branch from the left of vascular bundle *B* with another branch at a different level. At *D* two nodal branches of larger size pass to the right and left from bundle *B*. These are at a higher level than are those at *C*.

Bundle *A* branches as it enters the node, a smaller branch bundle

continuing through it without much interruption. At two points, however, nodal bundles join this smaller branch. In various parts of the microphotograph may be seen bundles of smaller size passing from one level to another after traversing across the node for some distance. It should be borne in mind that this is but a minute piece of a large node; when viewed with a binocular or examined with the naked eye, the vascular bundles with the branches given off in the node make a rather confusing network.

In fig. 4 there is shown a single vascular bundle, its course being deflected into the leaf. A single bundle, dissected out, is shown in fig. 3. Such bundles almost without exception give off a series of small branches at the point where they first bend in the node toward the leaf. This is always at the point of their entry or the lower edge of the node. The small branches thus given off enter the nodal complex and unite with the many other similar small branches to be found there.

### Summary

1. The vascular strands in *Zea mays* can readily be stained for study with methylene blue, as shown in fig. 1.
2. The stained bundles and the complex nodal structure can readily be freed from confusing parenchymatous tissues by retting.
3. Single vascular bundles seldom pass through more than two or three nodes without branching.
4. The nodal complex of small branches arises from vascular bundles at the point of their entry into the node and from smaller peripheral bundles.
5. The nodal complex is the result of division and subdivision of these small bundles, which later anastomose with those from other bundles.

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## CLEISTOGAMY IN TWO SPECIES OF DANTHONIA<sup>1</sup>

PAUL WEATHERWAX

(WITH PLATE VIII AND TEN FIGURES)

*Danthonia* is one of a number of genera of grasses known to have two kinds of spikelets, one kind in the ordinary terminal inflorescence, and another kind, seldom seen, in the axils of the leaves.<sup>2</sup> A comparative study of these two types of spikelet in *Danthonia spicata* (L.) Beauv. and *D. sericea* Nutt. has shown some other interesting features apparently not previously known.

### Material

*D. spicata* is widely distributed in the eastern part of the United States. In Indiana its clumps of curly leaves often stand out as a striking feature of the winter flora of clay hills so poor as to support little else in the way of a ground covering. Material for this study was collected in open fields and in the woods on poor clay soil north and east of Bloomington, Indiana, and supplemented with occasional specimens from other parts of the state. *D. sericea* is reported as occurring on sandy soil from Massachusetts southward to Florida. A typical habitat is the pine woods on red, sandy clay soil of the Piedmont region. Material was collected from several places in Georgia, chiefly near Athens and Unadilla. Both species were identified by careful comparison with the descriptions and with material from the same or similar localities, which had been identified by Dr. A. S. HITCHCOCK or Mrs. AGNES CHASE.

### *Danthonia spicata*

The flowering culm of *D. spicata* may vary from a few inches to as much as 3 feet in height (fig. 1). It bears a single terminal panicle, which has only a few short branches, and usually not more than ten or twelve spikelets. At the base of each culm is a cluster of three to six leaves, some having lived through the winter, the others being

<sup>1</sup> Publication no. 31 of the Waterman Institute for Scientific Research, Indiana University.

<sup>2</sup> CHASE, AGNES, Amer. Jour. Bot. 5:254-258. 1918.

of the current year's growth. In the axil of almost every one of the latter (fig. 1 *ab*), completely inclosed by the sheath, is a single spikelet. These spikelets are usually one-flowered (fig. 2); but higher on the culm, where the internodes are much longer (fig. 1 *ac*), the axillary spikelets often have as many as ten florets scattered along a greatly elongated rachilla (fig. 3).

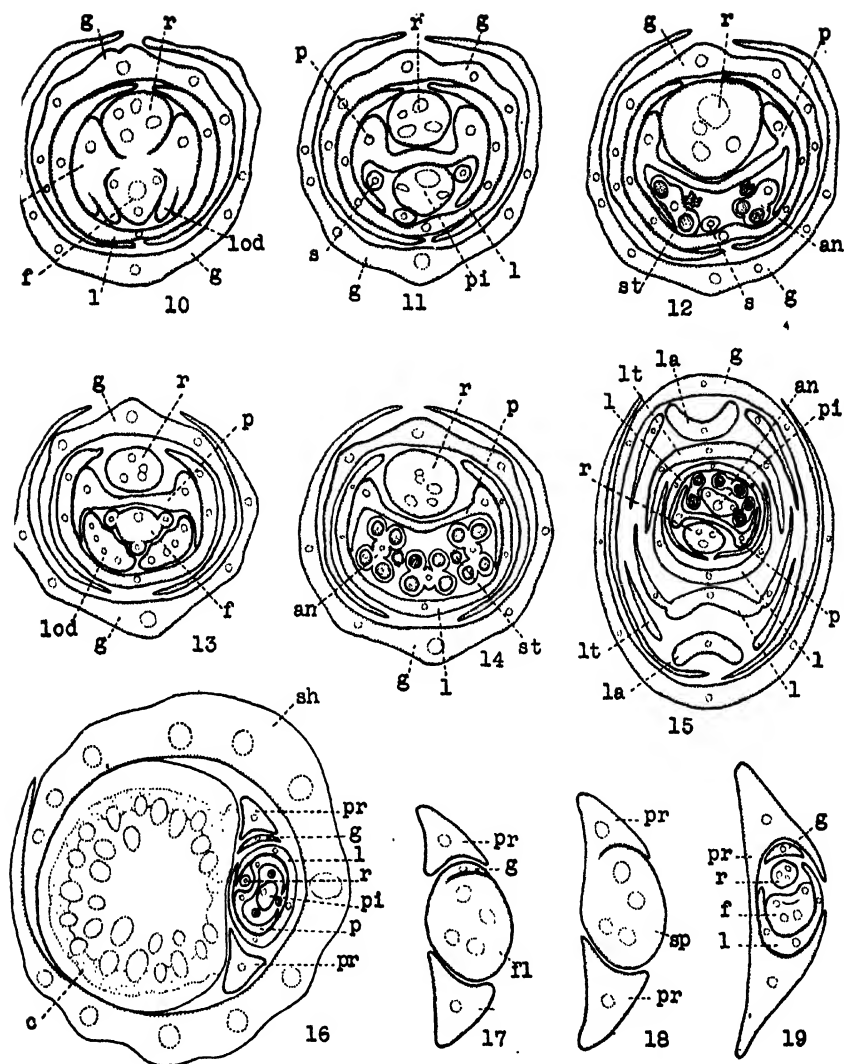
The spikelets in the terminal inflorescence (fig. 4) show no unexpected peculiarities in external appearance. Their lowest flowers are oldest, and the upper florets of each are usually imperfect or rudimentary. The glumes are usually as long as the rest of the spikelet.

The key to the peculiarities of the flower in both kinds of spikelets seems to be in the fact that the lodicules (figs. 6, 10 *lod*) are rudimentary and poorly supplied with vascular tissue, and the floret does not open at anthesis. The styles are short, and the stigmatic hairs are few and poorly developed. The anther is short and broad (figs. 5, 6). Each of its two loculi (fig. 12) contains only a few (40-60) pollen grains. Before the maturity of the pollen, the anthers become applied to the stigma, apparently adhering there or being held mechanically by the stigmatic hairs or by the closely investing lemma and palea (fig. 5). At anthesis they cannot be raised higher because the floret does not open, and the filaments, elongating as in other grasses, become coiled or otherwise distorted (fig. 6).

In such descriptions of *Danthonia* and its species as have been available, no special mention was found of the anther or lodicules, or of the nature of anthesis. The figure of the spikelet and floret of *D. spicata* in GRAY'S *Manual* (7th edition) is the only definite thing found on this point; it is most certainly wrong if my material is typical, for it shows anthers four times as long as wide, and distinctly exerted from the open floret on long filaments. It seems improbable, however, that this occurrence of cleistogamy should have escaped observers thus far.<sup>3</sup> It is most strikingly seen when developing or mature seeds are examined, the empty anthers being invariably found tangled in the hairs of the stigma.

The exact homologies of the parts of the axillary units (figs. 2, 3)

<sup>3</sup> Since this was written I have found that HACKEL (Nat. Pflanzenfam. II. 2:9) mentions *Danthonia spicata* in a short list of cleistogamous species.



FIGS. 10-20.—Figs. 10-12, transverse sections of terminal spikelet of *D. spicata* at different levels: g, glume; l, lemma; p, palea; r, rachilla; s, filament of stamen; st, stigma; an, anther; pi, base of pistil; lod, lodicule;  $\times 20$ ; figs. 13-15, transverse section of terminal spikelet of *D. sericea* at different levels: f, base of stamens and pistil; la, lt, awn and teeth of lemma; remainder of legend as in figs. 10-12;  $\times 20$ ; fig. 16, transverse section just above culm node in *D. spicata*, showing axillary spikelet in position: c, culm; sh, sheath of subtending leaf; pr, prophyllum; other parts of legend as in figs. 10-15;  $\times 15$ ; figs. 17, 18, sections of axillary spikelet (*D. spicata*) at different levels, showing relation of glume to lobes of prophyllum:  $\beta$ , base of floret; sp, base of spikelet; otherwise as in preceding figures;  $\times 20$ ; fig. 19, section of axillary spikelet of *D. sericea*, legend as in preceding figures.

are not clear. On the outside are two slender structures (figs. 2, 3, 16-18 *pr*) which might be regarded as glumes were they not associated with a third bract (figs. 16, 17 *g*), which also has the character of a glume. Tracing the vascular tissue has failed thus far to give any material aid.

The floret is essentially much the same as in the terminal spikelets. The lemma and palea are a little more indurated, and the former is awnless in some of the one-flowered spikelets, but usually awned in the upper florets of the elongated spikelets (figs. 2, 3). The pistil and stamens are identical in appearance with those of the terminal spikelets. No lodicules have been observed.

In both inflorescences the florets disarticulate from the rachilla, and the caryopsis remains inclosed in the lemma and palea. Fruits from the terminal spikelets are readily dispersed by the wind or other agencies; those from the axillary spikelets remain in the leaf sheaths and are scattered when the culm breaks away at maturity. In some cases the two types of disarticulated floret differ greatly in appearance, because of the variable nature of the lemma, but there seems to be no consistent difference in the caryopsis. Seeds from both sources germinate alike, and seedling plants observed until flowering, the second season after germination, are alike in appearance and vigor.

#### *Danthonia sericea*

Except for minor details, the individual plant of *D. sericea* is much like that of *D. spicata* in appearance. The terminal panicle often has as many as twenty spikelets. These do not differ materially from those of *D. spicata*. All the axillary units observed are one-flowered (fig. 7), however, and none have been found except at the two or three lowest nodes, at the very base of the culm. The outer part of each of these units, moreover, consists of a single bract (figs. 7, 19 *pr*), which seems to be equivalent to the two prominent lateral structures of the same organ in *D. spicata* (figs. 2, 3, 16-18 *pr*). The third glumelike scale is also present (fig. 19 *g*), but smaller than in *D. spicata*, seldom reaching higher than the base of the ovary. In the floral parts little difference between the species is seen except in the degree of development reached by the anthers and lodicules. In the axillary spikelets (fig. 9) the anthers are somewhat

longer and better developed than those of *D. spicata*. Lodicules are apparently not present. In the terminal spikelets the lower flowers have long, tetrasporangiate anthers (figs. 8, 14) and large, well developed lodicules (fig. 13), which do not function, however, at least not in the ordinary way. The upper flowers have bisporangiate anthers (fig. 15), and their lodicules are rudimentary if present at all. The anthers in the middle of the spikelet show various stages of degeneration of the ventral pair of sporangia; but in a few cases both sporangia on one side of the anther degenerate, leaving an asymmetrical structure.

### Interpretation

Most of the floral structures of both species are readily explained in terms of the ordinary morphology of grasses, but the identity of the bracts at the base of the axillary spikelet is not clear at present. Of the homology of the two structures in *D. spicata* with the one in *D. sericea* (*pr* in all figures) there can be little doubt; but their relation to the rest of the axillary branch is less evident. Two interpretations are possible:

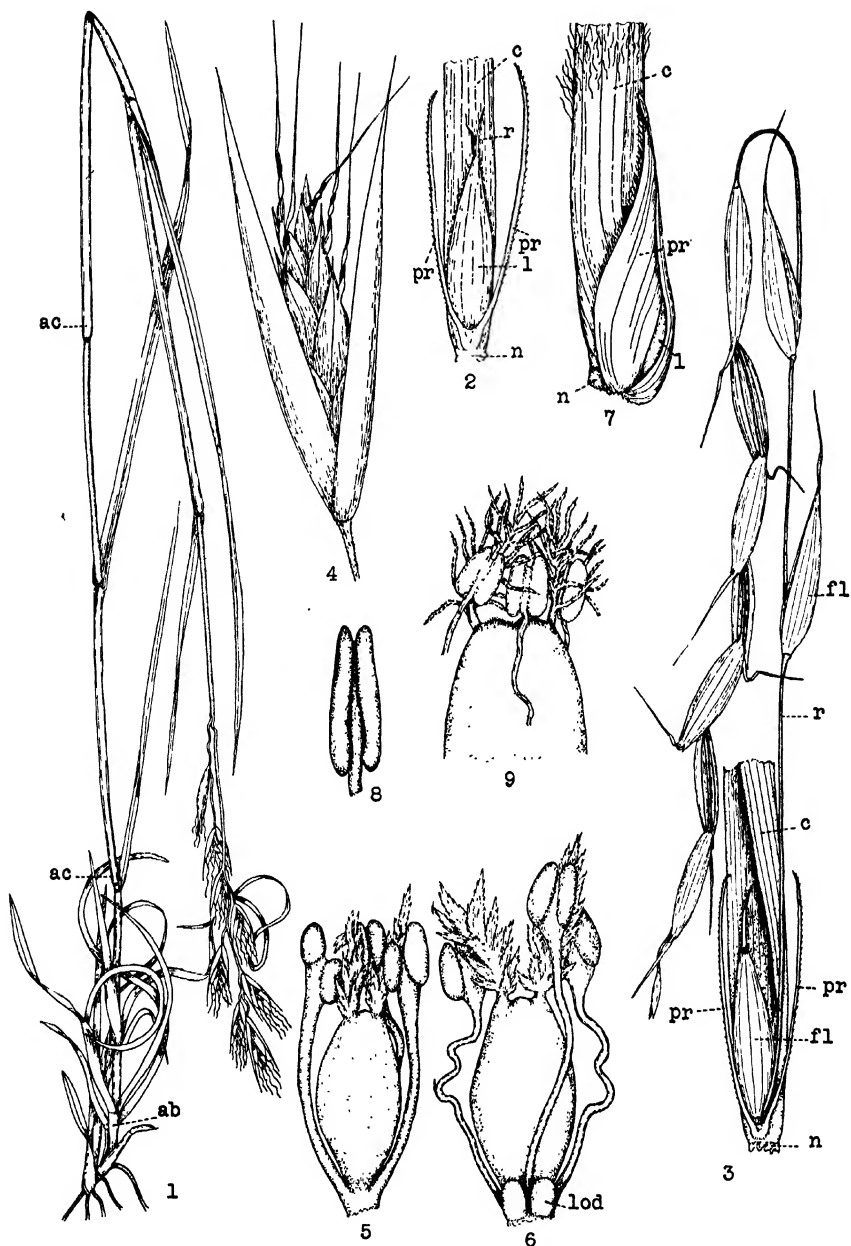
1. In *D. spicata* two separate glumes are present, and these are united into a two-keeled, two-nerved structure in *D. sericea*. The third very small bract in both species is an additional glume (or sterile lemma) such as occurs in *Uniola*, *Phalaris*, etc.

2. The axillary branch in each case bears a prophyllum, which is normal in *D. sericea*, but cleft to its base in *D. spicata*. The third scale is the second glume of the spikelet, as indicated by its position with reference to the lemma. The first glume is lacking.

The latter explanation is the same as that advanced by Mrs. CHASE in the article previously cited, except that she did not find the glume. It is regarded as the more reasonable, and it is in accord with it that the figures have been labeled. It would be materially strengthened by the finding of the other glume, or of an axillary unit in which there were two or more spikelets above the structure regarded as a prophyllum. The study of other species of *Danthonia*, or of other genera having similar peculiarities, will doubtless be helpful in interpretation.

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WEATHERWAX on DANTHONIA



## EXPLANATION OF PLATE VIII

FIGS. 1-9.—*Danthonia spicata*. Fig. 1, one flowering culm: *ab*, basal axillary spikelets; *ac*, axillary spikelets higher on culm;  $\times \frac{1}{2}$ . Figs. 2, 3, spikelets from positions *ab* and *ac* respectively, indicated in fig. 1;  $\times 4$ . Fig. 3, showing spikelet bent near middle: *pr*, lobes of prophyllum; *c*, culm; *l*, lemma; *r*, rachilla; *n*, node at which axillary unit is borne; *fl*, floret. Fig. 4, terminal spikelet;  $\times 4$ . Figs. 5, 6, flower from terminal spikelet, before and after anthesis: *lod*, lodicule;  $\times 30$ . Figs. 7-9, *D. sericea*. Fig. 7, basal axillary spikelet: *n*, node at which spikelet is attached; *c*, culm; *pr*, prophyllum; *l*, lemma;  $\times 4$ . Fig. 8, anther from one of the lower flowers of terminal spikelet;  $\times 20$ . Fig. 9, top of developing caryopsis, showing position of spent anthers;  $\times 20$ . In figs. 5, 6, and 9 the stigmas are shown very diagrammatically.



## STUDIES IN THE GENUS MENTHA

### 14. OXIDASES OF MENTHA PIPERITA

SAMUEL M. GORDON<sup>1</sup>

The manner in which cyclic compounds, especially those belonging to the so-called group of terpene compounds, are formed in the plant is as yet a field hardly beyond the speculative stage. Various theories have been proposed from time to time. Whether these theories fit the biochemical conditions is still a matter of conjecture, but they are interesting in at least providing a point of departure for experimental attack. The most satisfactory theoretical scheme which has been proposed is that of KREMERS (7). The outline proposed by KREMERS was intended to account for the relations found in three closely related botanical species of *Mentha*, *M. aquatica* L., *M. viridis* L., and *M. piperita* L. It satisfactorily accounts for most of the terpene bodies found in the volatile oils of these plants.

While the hypothesis in general clearly accounts for the biogenetic relations of the compounds present in the oil, much remains to be done along these lines. In the case of *M. piperita*, the problem of the menthol-menthone relations is still obscure. Menthol, both free and combined, forms over 50 per cent of the oil, while the content of menthone may vary between 10 and 15 per cent; yet the question as to which of these products is first formed is still a speculative one. Is menthol the first product formed, and then oxidized to menthone, or does a reduction process of menthone to menthol take place? To throw some light on this problem was the object of the investigation reported here.

In 1900, CHARABOT and coworkers (3) examined growing peppermint plants, grown in France, for the content and composition of the volatile oil isolated. They arrived at the following conclusions, which may be summarized briefly as follows: (1) At the beginning of the vegetation of the mint, the oil is rich in menthol, a small part of which is in the combined state. (2) Menthone exists only in

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minimal amounts at this period. (3) The menthone, which is but a small percentage of the oil at the beginning, increases with the vegetative process at the expense of menthol, the total menthol-menthone content remaining constant. (4) The oxidation takes place chiefly in the inflorescence. (5) The quantity of esters and terpenes increases, probably due to "diastase" (4).

In passing it might be mentioned that similar relations were found for a number of other volatile oil-bearing plants, which bear the peculiar alcohol-ketone and alcohol-aldehyde relation. In a summary of his researches CHARABOT states:

Alcohol formation takes place in the first phases, the second phase takes part in that period of the plant life in which the respiratory energy exceeds that of assimilation. The quantity of oxygen absorbed by the tissues at that period is very considerable, and by this process the alcohols are oxidized to aldehydes and ketones.

Sometime later KLEBER (6) examined oils from peppermint plants of different ages and found the reverse relations to hold true, that is, the oil from the younger plants contained a larger percentage of menthone than oils from older plants. Here a reduction and not an oxidation process is involved.

While nothing is said of the biochemical agents concerned in the oxidation process, it is assumed that enzymes of an oxidizing nature may have been a factor in the transfer of oxygen, or involved in the oxidation of menthol to menthone as assumed by CHARABOT. In 1912 BROOKS (2), tacitly assuming the experimental results of the French workers, reasoned that aldehydes and ketones are formed from the respective alcohols by a process of direct oxidation. However, while BROOKS obtained positive tests for catalase and peroxidases by the usual reagents, he was unable to oxidize menthol to menthone by the aid of oxidase isolated from the plant.

Quantitative determinations of the amounts of catalase and peroxidase were made during the summer of 1926, in order to learn something of the mechanism involved in the oxidation processes taking part in the life of the plant. Catalase was determined by the usual method of measuring the oxygen evolved from  $H_2O_2$  by a plant extract, while peroxidase was determined by the method of WILLSTÄTTER and STOLL (9).

### Experimentation

The *M. piperita* used in this investigation was obtained from a strain of plants which has been grown for a number of years, with necessary cultural precautions, at the gardens of the Wisconsin Pharmaceutical Experiment Station at Madison. A plot of about 15 feet square, from the regular peppermint crop, was placed at the writer's disposal by the superintendent of the gardens.<sup>2</sup> In all of

TABLE I

DATE	OXYGEN (CC.) LIBERATED, AFTER MINUTES						
	5	10	15	20	25	30	35
July 15 .....	36.10	48.3	48.9	48.9	.....	.....	.....
19 .....	38.10	44.2	44.9	44.9	.....	.....	.....
23 .....	55.85	59.6	59.8	60.1	60.1	.....	.....
August 2 .....	80.50	88.7	90.8	91.1	91.1	.....	.....
9 .....	67.90	78.5	87.9	91.6	93.8	95.0	96.5
16 .....	51.00	60.3	61.0	64.7	64.7	.....	.....
24* .....	55.80	60.7	63.5	63.5	.....	.....	.....
FLORETS ALONE USED							
August 2 .....	37.8	47.1	47.1	.....	.....	.....	.....
9 .....	37.6	46.2	46.3	.....	.....	.....	.....

\*Beyond this point it was difficult to obtain healthy samples of leaves, as the remainder of the field of mint was harvested and grasshoppers began destroying the plants.

these experiments not more than two hours elapsed between the time of harvesting and the determinations. The leaves were stripped from the stems, wrapped in waxed paper, and quickly transferred to the laboratory. Attempts were made to obtain as representative a sample as possible by picking leaves of different sizes and from different parts of a number of plants. Thus selected, the samples should yield a representative cross-section of the activity of the plant.

### CATALASE VALUES

A 0.5 gm. sample of cut leaves was ground to a fine pulp in a mortar, using dry, clean, sea sand. The ground sample was quickly transferred to the apparatus suggested by HAWK (5), 20 cc. neutral  $H_2O_2$  added, and the oxygen evolved collected and measured in a

<sup>2</sup> The gardens are under the immediate control of Dr. W. O. RICHTMANN, to whom I here express my thanks.

Schiff nitrometer. The volume of oxygen was reduced to standard conditions of temperature and pressure for purposes of comparison. A chloroform extract of the ground leaves did not liberate oxygen from hydrogen peroxide.

### PEROXIDASE VALUES

The peroxidase values were determined simultaneously with the catalase values described in the preceding section by the method of

TABLE II

DATE		PURPURUGALLIN PRODUCED (GM.)	"PURPURUGALLIN NUMBER"*
July	15 . . . . .	0.02950	3.387
	19 . . . . .	0.02930	3.363
	23 . . . . .	0.03000	3.432
August	2 . . . . .	0.02691	3.089
	9 . . . . .	0.05147	5.909
	16 . . . . .	0.01538	1.765
	24 . . . . .	0.00871	1.000
	29 . . . . .	0.00915	1.050
		FLORETS ALONE USED	
August	2 . . . . .	0.00861	0.988
	9 . . . . .	0.00860	0.988

\*The "purpurugallin number" as determined here is used in a different sense from the purpurugallin number of WILLSTÄTTER and STOLL. The basis of their results depends on a weighed quantity of highly purified enzyme; the "purpurugallin number" as used here is obtained by dividing the amount of purpurugallin by 0.00871, the smallest amount of purpurugallin obtained. They represent ratios rather than absolute values.

WILLSTÄTTER and STOLL. The test depends on the ability of the enzyme to oxidize pyrogallol to purpurugallin with hydrogen peroxide. The values are obtained by comparison with a standard solution of purpurugallin, containing 0.1 gm. in one liter of ether at 20° C.

### Discussion

An examination of the catalase and peroxidase values for the leaves shows that the amounts of these enzymes increase up to the flowering period, then suddenly drop. On the other hand, the activity of enzyme extracts from the florets alone is much below the values obtained for the leaves at any period. So far as the florets are concerned, the figures are entirely contrary to CHARABOT'S

statement that the seat of oxidation of menthol is in the inflorescence, if a direct oxidation is involved. The values for the leaf bear out CHARABOT's contention, however, since the leaves show highest enzymatic activity during the flowering period. This again is contrary to CHARABOT, who maintains that dehydration, that is, formation of esters and terpenes, takes place in the leaf. This phase of the reaction, however, can have no relation to the oxidizing enzymes. However, these results may be correlated with CHARABOT's contention by assuming that oxidation, if the oxidizing enzymes are involved, takes place in the leaf, and that the oxidized product, menthone, is transported to the flower, thus disturbing the equilibrium. In the introduction it was mentioned that when the florets were removed, very little menthone was found in the leaves by CHARABOT. From these results, it might be assumed that the leaf has a limited maximum capacity for retaining the oxidized product, which might inhibit the oxidation from being carried further, unless these products were removed. The function of the floret may then be considered as a storage medium for the menthone. In this way the oxidized products are carried from the leaf to the flower, lowering the amount of menthone in the leaf, and thus allowing further oxidation to proceed. By assuming such a mechanism of oxidation and transfer, the analytical results here reported may be brought into harmony with the data and contentions of CHARABOT.

While the peroxidases seem definitely to accelerate oxidation in biological process, the function of catalase is not definitely known. Two diverse views of the nature of catalase may here be presented. ABDERHALDEN (1) states, "the presence of catalase does not diminish the oxidizing powers of the other oxidizing enzymes." On the other hand, ROBERTSON (8) states, "Catalase is in fact a retarder of oxidations and not an accelerator, for it anticipates the action of peroxides and decomposes them, thus depriving them of their ability to transfer oxygen to oxidizing material. It is probably to be regarded as a controlling agent or check upon overactivity of oxidizing enzymes."

Another report will deal with attempts to oxidize menthol to

menthone by means of an extract of oxidizing enzymes from the plant, under a variety of conditions.

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# CURRENT LITERATURE

## BOOK REVIEWS

### How a tree grows

A little volume by SOMERVILLE<sup>1</sup> begins with the cell and the growing stem tip, and describes in rather simple terms the structure of the various organs of the tree. Buds receive rather careful attention, but leaves are rather superficially considered. The stem and its wood structure occupy the larger portion of the volume. The various elements of the wood are clearly described, and the descriptions are illustrated by a series of very good drawings. The identification of various timbers by microscopic examination is described, and again the illustrations are clear and well suited to the purpose they are expected to serve. Among other topics discussed are root structure and mycorrhiza, the origin and structure of the seed, the ascent of sap, the fall of the leaf, and the healing of wounds.

The entire discussion is of a type well suited to elementary studies in dendrology such as might be undertaken by junior college students. From the non-technical character of the language employed, the book may be read with interest and profit by amateurs interested in trees. The style is good, and in spite of the fact that the examples cited are mostly European species, it seems to meet a need in American schools in a successful and interesting manner.—G. D. FULLER.

### Palms of India

A RECENT VOLUME<sup>2</sup> undertakes to describe the indigenous and introduced palms growing in British India. The botanical descriptions of the various species include both the scientific and common names used by the English and by the native tribes of India, together with notes on seedling habits, distribution, and cultivation. In many instances more space is devoted to economic uses than to the more strictly botanical features of the species. The large number of plates and text figures tend to bring the trees more vividly before the reader, in spite of the fact that the quality of many of the plates leaves much to be desired. Five tribes are described, including in all 131 species.

An extensive bibliography and good index conclude the volume.—G. D. FULLER.

<sup>1</sup> SOMERVILLE, W., *How a tree grows*. pp. 212. figs. 112. Oxford Univ. Press, American Branch, New York. 1927. \$3.50.

<sup>2</sup> BLATTER ETHELBERG, *The palms of British India and Ceylon*. London, Oxford University Press. pp. xxviii+600. pls. 106. figs. 49. 2 maps. 1926. Printed at the Kanarese Mission Press, Mangalore, India. \$15.00.

## NOTES FOR STUDENTS

**Plant distribution.**—Although the present and the past distribution is critically known for a comparatively small percentage of the total number of plant families, genera, and species, this percentage constitutes a rather formidable group when the attempt is made to map each separately. The production of such a series of maps on a rather large scale is the ambitious aim of HANNIG and WINKLER.<sup>3</sup> It is certainly a task of no small difficulty, even with the assistance of such noted plant geographers as DIELS and SAMUELSSON, and the cooperation of a notable group of contributors. The latter are to include the most distinguished geobotanists of the day, if one is to judge from this first issue, which contains contributions by ENGLER, PAX, DIELS, VIERHAPPER, RIKLI, and HANNIG.

The general appearance of this publication recalls KARSTEN and SCHENCK's *Vegetationsbilder*, although it has an even larger page, 33×30 cm. Several of the ten maps in the first issue are of double-page size, and all are excellent in execution. The base map being in fine lines of blue, with the distribution limits in heavy black, the maps are easily read, while the high reputation of the contributors vouches for their accuracy. An explanatory text accompanies the maps. There is no doubt that this publication will be indispensable to all students of plant distribution.

In this first issue ENGLER maps the distribution of certain sections of the genus *Saxifraga*; PAX shows the areas occupied by the various subdivisions of *Acer* at present and during Tertiary times; DIELS maps *Casuarina*; VIERHAPPER, *Soldanella*; RIKLI, *Pinus pinea*; and HANNIG, *Genista angelica*. Not the least important feature of these contributions is extensive citations of the literature concerned.—G. D. FULLER.

**Taxonomic notes.**—EXELL<sup>4</sup> has published an account of the species of *Polygala* known in Angola, organizing the material in a way to reduce the difficulties of identification. It is of interest to note that of the 31 recognized species in this African country, 17 are endemic according to the present records, and only one species extends beyond Africa. One new species is described.

BROWN<sup>5</sup> has described a new genus (*Imilaria*) of Mesembryanthemeae from South Africa, which he regards as probably of hybrid origin from two crossing genera. He infers this from the intermediate characters it shows, a fact which he has recorded in the name.

<sup>3</sup> Die Pflanzenareale. Sammlung kartographischer Darstellungen von Verbreitungsbezirken der lebenden und fossilen Pflanzen-Familien, -Gattungen und -Arten. Edited by HANNIG and H. WINKLER with the cooperation of LUDWIG DIELS and G. SAMUELSSON. 1: heft 1. *maps 1-10*. 1926. Published by GUSTAV FISCHER, Jena. 7. 50 mk.

<sup>4</sup> EXELL, A. W., An enumeration of the species of *Polygala* in Angola. *Jour. Bot.* 65:339-347. 1927.

<sup>5</sup> BROWN, N. E., Two new Mesembryanthemeae. *Jour. Bot.* 65:348-349. 1927.



In continuation of his investigation of the flora of tropical Florida, SMALL<sup>6</sup> has described a new species of *Lechea* (Cistaceae), "which grows in the most southern island of scrub known on the eastern coast." He has also described<sup>7</sup> a new species of *Chamaesyce* (Euphorbiaceae), which grows in the pineland region of the Everglade Keys.

LEWTON<sup>8</sup> has described a new genus (*Shantzia*) of Malvaceae from Africa formerly referred to *Thespesia*. The name is in honor of H. L. SHANTZ, who as Agricultural Explorer obtained the seeds in Rhodesia in 1919. The plants grown from the seeds have been under observation for several years, with the final conclusion that they represent an undescribed genus.

In continuation of his revision of the grasses of Japan, HONDA<sup>9</sup> has published 6 new species in 5 genera, and also 5 new varieties, and 3 new names.

LEONARD<sup>10</sup> has published the result of his investigation of *Ruellia tuberosa* (Acanthaceae) and related forms. The forms considered are distributed in the West Indies and adjacent South America and the United States. Under *R. nudiflora* 8 varieties are recognized, 6 of which are new and 2 transferred from other species.

STANDLEY<sup>11</sup> has described 12 new species of plants from Central America in 6 genera, 7 of the species belonging to *Ardisia* (Myrsinaceae).

In continuation of his publication of Portuguese West African plants, GOSSWEILER<sup>12</sup> has described 4 new species of *Hippocratea* (Celastraceae).

GLEASON<sup>13</sup> has published his tenth contribution to the flora of northern South America. It includes 15 new species in 12 genera.

RYDBERG<sup>14</sup> has described three new species from the Blue Ridge region, in the following genera: *Hypericum*, *Kneiffia*, and *Stachys*.

SMALL<sup>15</sup> has described a new species of *Nyssa* from the pineland swamps of

<sup>6</sup> SMALL, JOHN K., A new pinweed from southern peninsular Florida. *Torreyana* 27:102, 103. 1927.

<sup>7</sup> ———, A new *Chamaesyce* from tropical Florida. *Torreyana* 27:104. 1927.

<sup>8</sup> LEWTON, F. L., *Shantzia*, a new genus of African shrubs related to *Gossypium*. *Jour. Wash. Acad. Sci.* 18:10-16. 1928.

<sup>9</sup> HONDA, M., Revisio Graminum Japoniae. XIV. *Bot. Mag.* 41:635-641. 1927.

<sup>10</sup> LEONARD, E. C., *Ruellia tuberosa* and a few of its close relatives. *Jour. Wash. Acad. Sci.* 17:509-520. 1927.

<sup>11</sup> STANDLEY, P. C., New plants from Central America. *Jour. Wash. Acad. Sci.* 17:520-528. 1927.

<sup>12</sup> GOSSWEILER, JOHN, Plants from Angola and Portuguese Congo. *Jour. Bot. Suppl.* 65:73-80. 1927.

<sup>13</sup> GLEASON, H. A., Studies on the flora of northern South America. X. *Bull. Torr. Bot. Club* 54:603-618. 1927.

<sup>14</sup> RYDBERG, P. A., New species from the Blue Ridge. *Toreya* 27:84-90. 1927.

<sup>15</sup> SMALL, JOHN K., A new *Nyssa* from Florida. *Torreyana* 27:92-93. 1927.

Florida. The author says that the specific name *ursina* "refers to the fact that the bears eat large quantities of the fruit in the fall and winter seasons." SMALL<sup>16</sup> has also described a new species of *Chamaesyce* which belongs to the endemic flora of the Everglade Keys.

COCKERELL<sup>17</sup> has described a new oak, *Quercus ulensis*, from the Eocene deposits in the Roan Mountains of Colorado.

In continuation of the contributions to the Flora of Siam by the Royal Botanic Gardens, Kew,<sup>18</sup> 26 new species are described, all but two of them being Leguminosae. The new genera are *Afgekia* (Galegeae) and *Nimiria* (Mimoseae).

NAKAI<sup>19</sup> has published the sixteenth part of the woody flora of Korea, describing the Araliaceae and Cornaceae. The text is in both Japanese and English, and is a very detailed presentation of the classification. The collection of plates is especially noteworthy, showing in detail the structures of all the species. The Araliaceae include 12 species, in 7 genera, 3 of the species being new. The Cornaceae include 8 species in 5 genera, a new tribe (Aucubeae) being established.

COKER<sup>20</sup> has described 17 noteworthy species of Basidiomycetes from North Carolina, in 12 genera, illustrated by 11 plates. Five of the species are new, belonging to the genera *Cyphella*, *Craterellus*, and *Amanita*.—J. M. C.

**Anatomy of variegated leaves.**—The nineteenth contribution to LINSBAUER'S *Handbuch der Pflanzenanatomie* is a discussion by KÜSTER of the anatomy of the variegated leaf. Although KÜSTER<sup>21</sup> urges the desirability of restricting the term variegated to those types of leaves in which the border between green and lighter areas is sharply marked, he devotes Part I of his treatise to a discussion of chlorosis, mosaic diseases, etc. (variegations in which the border is not sharply marked). In this part he considers the following topics: Form, position, and vertical distribution of light and green areas; histology; cytology; physiology; and etiology. The author takes the position that transmissible variegations (chloroses) are not the result of the action of pathogenic organisms, and that all attempts to invoke these as causes are based upon faulty observations and improbable assumptions.

<sup>16</sup> ———, A new *Chamaesyce* from Florida. *Torreya* 27:93-94. 1927.

<sup>17</sup> COCKERELL, T. D. A., A new oak from the Green River Eocene. *Torreya* 27:94-95. 1927.

<sup>18</sup> Contributions to the Flora of Siam. XXIII. Kew Bull. no. 9. pp. 374-395. 1927.

<sup>19</sup> NAKAI, T., Flora Sylvestica Koreana. XVI. Araliaceae and Cornaceae. Forestal Exp. Sta. Keijo, Japan. pp. 92. pls. 29. 1927.

<sup>20</sup> COKER, W. C., New or noteworthy Basidiomycetes. Jour. Elisha Mitchell Sci. Soc. 43:129-145. pls. 12-22. 1927.

<sup>21</sup> E. KÜSTER, Anatomie des panaschierten Blattes. *Handbuch der Pflanzenanatomie*, edited by K. Linsbauer. 8:1-68. figs. 54. Berlin: Gebrüder Borntraeger.

Part II, which comprises the larger part of the volume, is devoted to variegations with sharply defined areas. In addition to the topics discussed in Part I, the second part includes a discussion of distribution of variegations on the leaf blade and on the shoot, and developmental aspects and biology. An appendix is devoted to anthocyanin variegations. This excellent volume is concluded with a literature list of 162 titles, in which the American literature is included.—G. K. K. LINK.

**Chemical nature of enzymes.**—A problem of very great interest and importance in biology, and one which has long resisted solution, is the chemical nature of enzymes. Enzymes have been regarded by some as carbohydrate in nature, and by some as protein. Most of the recent work has supported the latter view. It has been suggested that a particular group in a complex molecule is responsible for enzyme activity, and FALK has made the suggestion that different groups in a complex molecule may be responsible for various enzyme activities.

SUMNER<sup>22</sup> seems to have shown definitely that the enzyme urease,<sup>23</sup> of *Canavalia ensiformis*, is a protein of the globulin group. He has obtained the protein in the form of octahedral crystals, which the microscope shows are practically uncontaminated with foreign material. They showed much greater urease activity than the purest urease preparations previously obtained by the author without crystallization. In order to get a solution of urease it was necessary to dissolve the crystals; if solvents were used, which did not dissolve the crystals, the solutions obtained showed little or no urease action. Chemical tests showed that the crystals were purely protein, and previous work on urease has shown that it behaved as a protein when treated with various reagents. Tests showed that the crystals were nearly free of ash, and this too agreed with previous work on preparations of the enzyme.

It will be interesting to see whether the urease of other plants is the same as that of the jack bean, and also whether other enzymes can definitely be shown to be protein in nature. SUMNER's work should stimulate much further investigation along this line.—S. V. EATON.

**Fossil conifers of western America.**—MASON<sup>24</sup> has given an interesting account of the fossil record of conifers in the Pacific Coast region. The coniferous forest of the Pacific Coast is of great interest, on account of its endemic features. The species described, when considered in connection with the existing coniferous flora, seem to justify the conclusion that the presence of the endemic elements in the California region are relicts from floras that were widespread over much of the western part of North America.—J. M. C.

<sup>22</sup> SUMNER, J. B., The isolation and crystallization of the enzyme urease. Jour. Biol. Chem. 69:435-441. 1926.

<sup>23</sup> ———, The recrystallization of urease. Jour. Biol. Chem. 70:97, 98. 1926.

<sup>24</sup> MASON, H. L., Fossil records of some West American conifers. Publ. 346, Carnegie Inst. Wash. pp. 139-158. pls. 1-5. 1927.

# THE BOTANICAL GAZETTE

*April 1928*

## LODGING IN OATS AND WHEAT

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 377

F. A. WELTON

(WITH THIRTEEN FIGURES)

### Introduction

Between erect and lodged grain no arbitrary line can sharply be drawn, since the one condition merges gradually into the other. In this paper the term erect is applied to grain which at harvest time stands upright, or nearly so. By lodged grain is meant that which has partly or completely lain over on the ground and which may have become more or less tangled. In some lodged grain the culms simply lie over on the ground without any perceptible bend at the base of culm; in other cases there is an abrupt bend in the culm at one of the lower nodes, in which case a field presents an appearance like that shown in fig. 1. Occasionally the bend occurs at one of the upper nodes, and then a situation develops like that presented in fig. 2.

Lodging may affect very materially both the quantity and quality of the grain, the degree of loss depending largely on the completeness of the falling, the state of development at which it occurs, and the subsequent weather conditions. Frequently the loss due to imperfect development of kernels may range from five to ten or more bushels per acre.

### Historical

The work heretofore done on lodging may, in a broad way, be grouped into three classes: observational, anatomical, and chemical.

OBSERVATIONAL.—THÄER (32), an English physician and agriculturist, was among the earliest to take note of lodging in wheat, and he attributed it to rich ground, abundant manuring, poor plowing, thick sowing, and sometimes to a diseased condition. KUHN (18), MAYER (21), VON SEELHORST (37), HOUTMAN (8), HALL (6), and many others have observed that lodging is often associated with the presence of excessive nitrates or with rich ground in general. The various constituents contained in fertilizers have also been mentioned. THORNE and HICKMAN (33), for example, observed more



FIG. 1.—Field of lodged rye, showing most of the culms bent over abruptly near one of the lower nodes.

lodging in wheat from the use of a mixture of phosphate and nitrate of soda than from the use of either alone. MURRAY (23) observed less lodging in rye where the fertilizers contained some potash. HARCOURT (7) attributed the difference observed in a field of oats to the presence of more lime and less nitrogen in the part that remained erect. In an experiment with oats in Scotland (2), a top dressing of nitrate of soda increased lodging, and common salt appeared to be more efficient than did potash as a preventative.

ANATOMICAL.—LIENAU (20), VAGELER (36), ALBRECHT (1), MOLDENHAUER (22), GARBER and OLSON (5), PURVIS (27), and others have studied lodging in relation to the anatomical structure of the plants. Most of these workers failed to find any relationship be-

tween the anatomical structure and lodging. GARBER and OLSON, however, concluded that thinness of cell walls seemed to be associated with lodging.

**CHEMICAL.**—Weakness of straw has been attributed to lack of various elements, particularly silicon and potassium. That lack of silicon is responsible for lodging seems first to have been suggested by DAVY (3), who, as early as 1789, discovered that in the epidermis of rattan there is a sufficient quantity of flint to produce light when



FIG. 2.—Unusual type of lodging in wheat, showing many of the culms bent over abruptly near first or second node below the head.

struck by steel. As a result of this and other observations, he conducted certain experiments from which he concluded that siliceous earth is common in the epidermis of hollow plants, and that it serves as a support.

LIEBIG (19) also believed that weakness of culms was due to lack of an abundance of silicon. He ascribed the strength of all stems of the grass family to silicate of potash. The importance of silicon was emphasized also by KUHN (18), SWIECICKI (31), and KOHL (15). As a result of the work of SACHS (30), KNOP (13), JODIN (9), PIERRE (26), MAYER (21), and others, the importance of silicon began to be questioned. As late as 1916, however, DOUGLAS (4) gave lack of

silicic acid as one of many causes for lodging in sugar cane. Perhaps its presence renders the cell walls more resistant to attacks of insect pests and fungous diseases, for JULIEN and DUPONT (10) reported a case in which lodging of wheat was thought to be due to an infestation of two fungous diseases.

Straw weakness has also been attributed to lack of lignification in the supporting tissues in the culms. SACHS (30) observed that such a situation usually develops where the stand of plants is too thick, the culms therefore shading one another unduly and thus taking on the diseased condition of etiolation. Lack of sufficient light was considered as an important factor by KOCH (14), KRAUS (16), RIVERA (29), WIESSMAN (39), PERCIVAL (25), PALLADIN (24), and others.

**SUMMARY.**—From the foregoing review it is clear that lodging has long been associated with hypernutrition, particularly of a nitrogenous nature. It is also apparent that many have considered it as related to reduced light, but no suggestion has been given as to how these two factors may interact with each other to bring about such a condition. The importance of silica from the standpoint of straw strength seems to have fallen into disrepute, by reason of the fact that plants have been found to make apparently normal growth even to maturity in nutrient solutions free from silicic acid. In the development of the silicic acid theory, the fact seems to have been overlooked that much of the silica found in the grasses is contained in the leaves rather than in the culms.

### Object

In a broad way lodging may be said to result from two sets of causes: (1) the interaction of those environmental factors which make for the development of weak stems; and (2) external forces which exert no influence on the structure of the stems but which cause lodging through mechanical impact, such as violent wind, rain, or hail storms. For obvious reasons this discussion is not concerned with the latter set of causes. The purpose of this paper has been to ascertain the environmental conditions responsible for the development of weak stems in oats and wheat, and to trace the chain of se-

quence from the non-lodged to the lodged condition through the internal responses produced by the interaction of the various factors involved.

### **Material and methods**

For this study there were used mainly pure line strains of oats and wheat. Some of the material was grown in pots in a greenhouse, and some (the major part) under field conditions.

For a study of the physical characteristics of lodged and non-lodged grain, samples of fully developed culms were gathered from definite areas in general fields, shortly before harvest. From these the stand of plants was determined, also the proportion of straw and grain. Other culms were gathered at the heading stage while the plants were green, and on these the diameter and breaking strength of the culms were measured.

For the chemical studies, 100 gm. samples of culms were gathered at heading time and preserved in alcohol. The samples of so-called lodged culms were composed in part of culms partially lodged and in part of erect culms selected from the border of lodged areas. Completely prostrate culms were avoided because it was thought that the bend in such stems might interfere with the normal translocation of nutrients and hence alter the composition. The samples of erect or normal grain were selected in regions where all the surrounding culms were standing erect. All the samples consisted of the lower third of the culms from which the leaves had been removed. The stems were cut into pieces one-half to one inch in length, placed in wide-mouthed, ground-glass stoppered bottles, and then covered with alcohol; the strength of the final solution, including the moisture contained in the stems, being about 70 per cent. After heating about one hour at approximately 78° C., the material was set aside and allowed to stand for at least two months. On these samples analyses were made for moisture and dry matter, nitrate and total nitrogen, and various carbohydrate compounds including cellulose, lignin, and sugars of different kinds.

Microchemical tests were made on fresh material for nitrates, free-reducing substances, cellulose, lignin, and to a less extent for some of the mineral substances such as potassium and calcium.



For the anatomical study a few typical culms of green stems were selected at heading time and preserved in a formalin-alcohol solution. Freehand sections were made and stained in safranin and light green.

## Results

### FIELD SURVEY

Samples of lodged and non-lodged oats were gathered from the field at harvest time in 1920, 1921, 1922, 1923, and 1924. In each year ten samples of each kind were selected. Each sample represented the harvest from an area one yard square. In 1920 the

TABLE I  
PERCENTAGE OF STRAW IN ERECT AND LODGED OATS

1920 OHIO 6222		1921 OHIO 201		1922 OHIO 201		1923 OHIO 201		1924 OHIO 201		
Erect	Lodged	Erect	Lodged	Erect	Lodged	Erect	Lodged	Erect	Lodged	
62.1	77.3	64.8	75.2	92.6	87.7	60.5	64.5	61.1	67.2	
59.0	79.6	62.9	76.4	94.4	86.5	57.0	73.8	60.6	68.0	
61.8	81.8	64.1	74.1	93.7	82.2	60.0	62.4	58.4	64.0	
63.6	85.5	65.0	74.4	90.6	90.3	59.9	68.9	59.9	65.9	
60.2	79.6	65.5	73.5	92.1	91.6	62.8	67.1	62.7	64.3	
59.1	93.2	69.8	75.1	93.4	86.9	59.3	73.1	60.6	64.3	
61.9	90.8	68.8	78.1	84.9	92.5	65.4	68.6	58.1	67.6	
60.6	95.9	68.0	75.9	70.7	76.4	60.8	71.1	56.7	62.4	
59.8	93.3	66.5	74.3	92.2	92.8	64.1	65.3	53.0	63.7	
61.4	94.4	66.4	77.6	92.3	91.8	58.9	65.4	58.4	70.2	
Average....	60.9	87.1	66.2	75.4	89.7	87.7	60.5	67.7	59.0	65.8

samples consisted of a pure line strain of the Improved American variety, Ohio 6222; in the other years they consisted of the Ohio 201, a selection from the Siberian variety. After weighing, each sample was threshed and the proportion of grain and straw calculated. The percentage of straw found in each individual sample of erect and lodged grain and the average of the ten samples of each kind for each year are given in table I. From this table it may be noted that the average percentage of straw was higher in the lodged than in the erect grain in every year except 1922. In that year a violent wind and rain storm caused most of the field to lodge, and this rendered difficult an accurate separation of the erect from what would probably have lodged under normal conditions.

Square yard areas of erect and lodged wheat were gathered in a similar way at harvest time in the years 1919, 1920, 1921, 1923, and 1924. In 1919 the samples were gathered from two varieties, Gladden and Fulhio; in 1920 from the Fultz and Fulhio; and in each of the other years from one variety, the Fulhio. With the exception of the Fultz these varieties are pure lines. After weighing and threshing, the percentage of straw was calculated; and the percentage of straw for each sample and the average percentage for each class for each year are given in table II. From this table it may be noted that the

TABLE II  
PERCENTAGE OF STRAW IN ERECT AND LODGED WHEAT

1919				1920				1921 FULHIO		1923 FULHIO		1924 FULHIO	
GLADDEN		FULHIO		FULTZ		FULHIO		Erect	Ldg.	Erect	Ldg.	Erect	Ldg.
Erect	Ldg.	Erect	Ldg.	Erect	Ldg.	Erect	Ldg.						
71.4	80.7	69.6	73.4	52.4	76.8	61.2	73.5	69.9	79.2	66.9	73.7	56.0	67.2
68.0	85.3	72.5	74.2	70.5	75.4	63.0	71.8	78.8	75.5	63.1	76.0	63.3	72.9
74.6	79.3	70.3	75.3	68.7	75.2	65.3	72.2	76.1	72.4	63.1	70.3	62.9	74.0
71.5	78.8	72.4	74.1	69.8	75.5	60.6	70.5	79.2	81.1	61.9	71.7	69.1	65.3
71.2	83.3	71.4	74.8	70.2	74.1	61.4	72.5	70.4	72.5	61.9	70.1	70.1	75.2
70.9	81.5	72.7	79.2	69.3	73.7	62.2	74.1	69.1	71.1	85.9	73.3	68.1	66.9
71.7	86.9	69.5	74.7	71.2	81.1	64.5	71.8	69.1	70.3	67.8	71.9	60.8	73.8
70.4	73.5	68.2	71.6	69.4	80.1	67.9	71.3	68.9	73.9	69.6	74.2	67.9	71.8
71.0	74.0	69.3	73.5	70.2	78.2	65.7	71.7	68.9	69.7			76.0	72.7
71.8	87.2	68.6	74.9	69.6	80.2	66.1	70.4	70.5	71.8			65.6	66.1
Average.....				71.2	81.0	70.4	74.5	68.1	77.0	63.7	71.9	72.1	73.7
				67.0	72.6	66.0	70.6	67.0	72.6	66.0	70.6		

average percentage of straw was higher in the lodged than in the erect grain in every year and in all the varieties. A part of the higher percentage of straw is due probably to a tendency toward the development of slightly longer heads in lodged than in erect grain. Measurement of the length of 1000 heads each of lodged and erect oats gave an average length of 275.8 and 255.1 mm. respectively. Although longer, the lodged heads were lighter, for the average weight per head was 1700 and 1890 mg. for the lodged and erect heads respectively. The lighter weight is due to the development of more shriveled kernels in lodged plants, and this is reflected in the weight per bushel of the threshed grain. In a field of oats, a part of which

lodged about heading time, the weight of seeds per bushel grown on lodged and erect plants was 22 and 33 lb. respectively.

In wheat also the lodged heads are longer but lighter. The average length of 1000 heads each of lodged and erect wheat was found to be 75.0 and 72.1 mm. respectively. The average weight per head of the same two lots of lodged and erect wheat was 830 and 900 mg. respectively.

The higher percentage of straw in lodged plants is due chiefly to the fact that in such the number and length of culms are greater

TABLE III  
NUMBER OF CULMS PER SQUARE YARD IN ERECT AND LODGED OATS

1920 OHIO 6222		1921 OHIO 201		1922 OHIO 201		1923 OHIO 201		1924 OHIO 201		
Erect	Lodged	Erect	Lodged	Erect	Lodged	Erect	Lodged	Erect	Lodged	
266	344	214	221	163	183	216	222	226	268	
285	366	129	244	202	185	211	201	188	287	
287	322	178	218	208	240	175	220	169	243	
274	333	158	222	196	238	221	233	180	262	
263	323	157	215	224	232	213	276	175	233	
275	343	156	313	167	218	205	230	162	252	
258	316	226	179	194	228	202	234	160	286	
272	318	181	197	175	199	246	214	143	270	
315	321	187	246	204	227	228	268	137	289	
273	331	204	248	200	231	222	240	179	277	
Average....	277	329	179	230	193	218	213	233	172	267

than in erect ones. That the number of culms is greater is shown by counts made on the same samples of oats and wheat for which the percentages of straw were given, as indicated in tables I and II. The counts were made before the grain was threshed, and the results obtained are recorded for the oats in table III and for the wheat in table IV. From table III it may be noted that the average number of culms was greater in the lodged than in the erect oats in each of the five years, the difference ranging from 16 to 74 per cent.

The denser stand of lodged grain is due probably to a relatively higher moisture and nitrate content of the soil, for almost invariably lodged grain is found on the lower and more fertile land, in depressions, ravines, etc., rather than on knolls, hills, or upland in general.

That relatively high moisture content of the soil tends to induce tillering was shown by a pot test conducted in the greenhouse during

the winter of 1919-1920. In this test two lots each of 42 one-gallon jars were filled with an equal quantity of rich compost, to which subsequently had been added and thoroughly mixed with it a liberal application of both lime and acid phosphate. In each jar four seedlings of pure line wheat, Ohio 13384, were planted January 9, 1919. The two lots of jars were treated alike in all respects except in the amount of water given; one lot being kept at optimum minus, the other at optimum plus. The average number of culms produced in

TABLE IV

NUMBER OF CULMS PER SQUARE YARD IN ERECT AND LODGED WHEAT

1919				1920				1921		1923		1924		
GLADDEN		FULHIO		FULTZ .		FULHIO		FULHIO		FULHIO		FULHIO		
Erect	Ldg.	Erect	Ldg.	Erect	Ldg.	Erect	Ldg.	Erect	Ldg.	Erect	Ldg.	Erect	Ldg.	
462	561	460	577	407	624	302	609	436	610	555	597	483	799	
473	695	390	540	406	579	282	519	445	582	558	513	595	763	
591	678	429	535	406	576	367	711	460	605	498	714	547	666	
480	560	421	667	404	592	274	473	493	617	565	493	564	545	
437	653	406	537	389	613	211	645	478	500	608	590	559	616	
476	645	380	582	394	535	282	667	454	539	463	510	444	752	
480	688	577	628	380	658	424	425	471	548	544	675	536	474	
429	554	420	477	446	631	401	490	440	484	410	779	466	669	
405	513	441	615	391	685	348	497	461	472			539	625	
393	651	482	540	460	644	370	504	447	485			492	738	
Average.....	462	619	440	569	408	614	318	554	458	544	525	609	523	665

the optimum minus and optimum plus jars was 7.4 and 8.5 respectively at the jointing stage. Many of the smaller culms died and withered away as the plants developed.

The addition of nitrates also tends to increase the amount of tillering. In a pot tested with wheat conducted in the greenhouse during the winter of 1920-1921, nitrate of soda was applied at different dates, the primary object being to note the effect of such applications on the quality of the grain. Incidentally the amount of tillering was noted. The wheat to which the nitrate of soda was applied averaged 3.0 culms per plant, while the untreated averaged 2.2 culms. A similar test made in the winter of 1921-1922 gave 2.7 culms and 2.0 culms per plant for the treated and untreated wheat respectively.

As a rule the straw of lodged grain is relatively soft and pliable as compared with that of erect grain, thus suggesting a variation in chemical composition. Accordingly samples grown under various conditions favorable for non-lodging and lodging were selected for chemical analysis.

#### CHEMICAL COMPOSITION

HIGH AND LOW GROUND.—Two samples each of oats and wheat grown in general fields were selected in 1924. One sample of each was selected from among erect grain; and one of each from among

TABLE V

COMPOSITION OF OAT AND WHEAT CULMS GROWN IN A GENERAL FIELD ON LOW AND HIGH GROUND IN 1924

MATERIAL	PERCENTAGE			
	OATS		WHEAT	
	Low ground (rich)	High ground (poor)	Low ground (rich)	High ground (poor)
Moisture.....	76.400	68.880	78.450	69.380
Dry matter.....	23.600	31.120	21.550	30.620
Nitrate nitrogen.....	0.003	0.011	0.001	0.002
Total nitrogen.....	0.072	0.068	0.082	0.053
Free reducing sugars.....	1.742	2.802	2.439	2.091
Disaccharides.....	0.174	4.051	1.684	9.932
Polysaccharides.....	3.854	5.221	4.201	5.484
Cellulose.....	3.725	3.259	3.379	3.021
Lignin.....	10.171	10.884	6.866	6.471
Total carbohydrates.....	19.666	26.217	18.569	26.999

that which was badly lodged. The lodged areas were located on relatively low ground. Naturally, such ground is more fertile and contains more moisture than the higher ground, for it receives the seepage from the latter. The distance was a matter of a few yards only in most cases. The results of the analysis are recorded in table V. From the table it may be noted that in both oats and wheat the culms of the plants grown on the low ground contained more moisture, slightly more total nitrogen, less dry matter, and less total carbohydrates than did those grown on the relatively high ground.

SAND-SOIL-MANURE TEST.—In order to study the effect of more extreme conditions of fertility on lodging, both oats and wheat were

grown in soils artificially prepared and representing wide variations, particularly as regards nitrates. The poor soil consisted of one part by measure of good silt loam mixed with three parts common creek sand. The sand had previously been passed through an ordinary sand screen in order to remove large stones and other foreign materials. The rich soil was made by mixing with the silt loam some well rotted manure in the proportion of one part by measure of soil and three parts of manure. The two classes of artificial soil were prepared to a depth of 7 inches, ordinary plow depth, and each embraced an area of one square yard. They were arranged one on

TABLE VI

EFFECT OF FERTILITY ON COMPOSITION OF OAT AND WHEAT CULMS, 1922

MATERIAL	PERCENTAGE					
	OATS			WHEAT		
	Sand $\frac{1}{2}$ Soil $\frac{1}{2}$	Soil	Soil $\frac{1}{2}$ Manure $\frac{1}{2}$	Sand $\frac{1}{2}$ Soil $\frac{1}{2}$	Soil	Soil $\frac{1}{2}$ Manure $\frac{1}{2}$
Moisture.....	74.039	72.555	80.670	67.711	69.941	74.966
Dry matter.....	25.961	27.445	19.330	32.289	30.059	25.034
Nitrate nitrogen.....	0.002	0.004	0.018	0.000	0.001	0.021
Total nitrogen.....	0.076	0.062	0.080	0.154	0.138	0.197
Free reducing sugars.....	2.803	2.090	1.178	1.443	1.443	0.661
Disaccharides.....	0.000	0.000	0.210	3.283	2.375	1.468
Polysaccharides.....	4.587	4.925	2.994	5.599	5.314	4.163
Cellulose.....	4.443	4.957	3.249	4.468	4.647	4.277
Lignin.....	8.776	8.982	5.894	10.460	10.408	7.981
Total carbohydrates.....	20.609	20.954	13.525	25.253	24.187	18.550

either side of an equal area of the natural soil, the Wooster silt loam, and were separated by one-inch boards. The three classes are designated in this paper as sand-soil-manure. Soils of these three grades were prepared for the growing of both oats and wheat. Crops of each were harvested and sampled for analysis in 1922, 1923, and 1924. The results of the analysis are given in tables VI, VII, and VIII.

From the tables it may be seen that in both oats and wheat and in all three years the plants grown in the manure, as contrasted with those grown in the sand or soil, were in general high in moisture and consequently low in dry matter and in total carbohydrates, including polysaccharides, cellulose, and lignin. They were also higher in

nitrate nitrogen and total nitrogen. Nitrate determinations made on the three classes of soil in 1923 showed 2.4, 3.9, and 135 parts per million in sand-soil-manure respectively.

TABLE VII

EFFECT OF FERTILITY ON COMPOSITION OF OAT AND WHEAT CULMS, 1923

MATERIAL	PERCENTAGE					
	OATS			WHEAT		
	Sand $\frac{1}{2}$ Soil $\frac{1}{2}$	Soil	Soil $\frac{1}{2}$ Manure $\frac{1}{2}$	Sand $\frac{1}{2}$ Soil $\frac{1}{2}$	Soil	Soil $\frac{1}{2}$ Manure $\frac{1}{2}$
Moisture.....	73.780	72.920	77.340	71.760	73.820	79.230
Dry matter.....	26.220	27.080	22.660	28.240	26.180	20.770
Nitrate nitrogen.....	0.021	0.017	0.058	0.003	0.006	0.066
Total nitrogen.....	0.180	0.181	0.357	0.176	0.173	0.278
Free reducing sugars.....	1.918	2.235	1.924	2.016	2.396	0.783
Disaccharides.....	0.596	1.514	2.001	5.359	3.152	0.954
Polysaccharides.....	6.064	5.733	4.115	5.653	5.332	3.960
Cellulose.....	3.315	3.342	2.550	3.111	3.085	2.977
Lignin.....	7.332	7.667	5.850	6.771	7.078	6.002
Total carbohydrates.....	19.225	20.491	16.440	22.910	21.043	14.676

TABLE VIII

EFFECT OF FERTILITY ON COMPOSITION OF OAT AND WHEAT CULMS, 1924

MATERIAL	PERCENTAGE					
	OATS			WHEAT		
	Sand $\frac{1}{2}$ Soil $\frac{1}{2}$	Soil	Soil $\frac{1}{2}$ Manure $\frac{1}{2}$	Sand $\frac{1}{2}$ Soil $\frac{1}{2}$	Soil	Soil $\frac{1}{2}$ Manure $\frac{1}{2}$
Moisture.....	76.060	77.080	80.230	59.000	69.800	79.380
Dry matter.....	23.940	22.920	19.770	41.000	30.200	20.620
Nitrate nitrogen.....	0.001	0.005	0.123	0.001	0.003	0.052
Total nitrogen.....	0.048	0.044	0.215	0.095	0.062	0.116
Free reducing sugars.....	1.670	1.771	0.537	1.916	2.129	1.045
Disaccharides.....	2.918	2.004	1.670	9.584	7.860	1.103
Polysaccharides.....	4.498	4.273	3.419	9.144	5.452	3.867
Cellulose.....	3.133	3.270	2.713	4.748	3.030	3.235
Lignin.....	9.540	8.919	6.535	11.896	7.890	7.180
Total carbohydrates.....	21.759	20.237	14.874	37.288	26.361	16.430

In general the stems of the plants grown in the soil, and especially those grown in the sand, were small and tough, while those grown in the manure were much darker in color. In each year the oats and

wheat grown in the manure lodged, or showed a strong inclination to lodge, while those grown in the soil and sand stood perfectly erect.

On the manure more and longer culms developed, thus resulting in a greater percentage of straw. The heavier growth produced more shade, and the difference in the intensity of this at the base of the culms, as measured by RIDGEWAY'S (28) chemical photometer, is indicated in table IX.

The percentages refer to the amount of oxalic acid decomposed under the influence of the light that struck the lower part of the

TABLE IX  
INTENSITY OF LIGHT AMONG OAT AND WHEAT PLANTS GROWN  
ON RICH AND POOR SOIL

TREATMENT	PERCENTAGE	
	Oats	Wheat
Sand.....	100	100
Soil.....	20	12
Manure.....	0	4

culms growing in the different kinds of soil. The amount decomposed among the plants growing in the sand was recorded as 100 per cent. The proportionate amount decomposed among the plants grown in the soil and manure was computed and recorded in percentage also. The percentages, therefore, represent not the absolute but the relative amount of light surrounding the lower part of the culms.

The low intensity of light among the plants grown in the manure may have been responsible in part for the relatively low carbohydrate content, because such conditions are not conducive to maximum carbon assimilation.

SHADING.—In order to discover the effect, if any, of reduced light on the composition of the stems, small areas of oats and wheat growing in general fields were covered with cheesecloth. Frames about 12 feet square and 5 feet high, as shown in fig. 3, were constructed and covered with a single layer of cheesecloth, ample provision being made for the free circulation of air. The frames were placed in position before the oats and wheat came into head, and were allowed to remain there until harvest. This was done in four



seasons, 1921, 1922, 1923, and 1924. In 1923 and 1924 samples of oats and wheat culms grown inside and outside the cheesecloth frames were taken at heading time. The analysis of the 1923 samples is given in table X, and of the 1924 samples in table XI.

From these tables it may be seen that a marked reduction in dry matter and total carbohydrates was found in the shaded oats and wheat in both years. The reduction occurred principally in the disac-

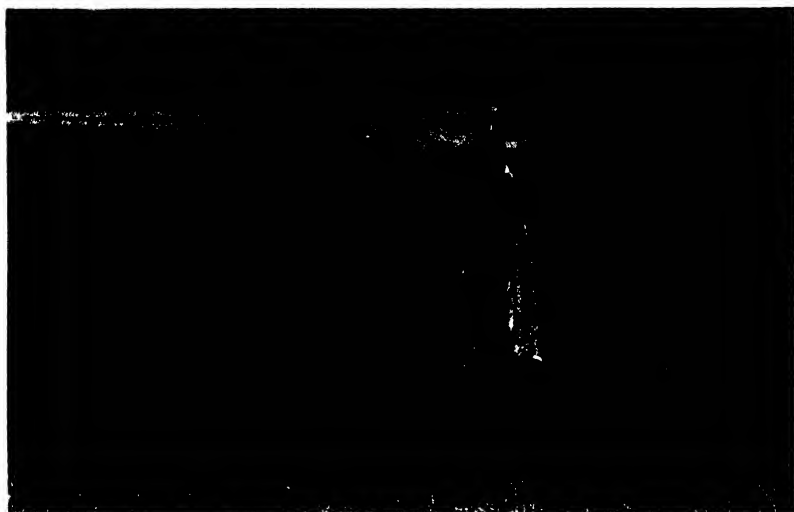


FIG. 3.—Shaded and unshaded wheat

charides, polysaccharides, and lignin, it being most marked in the disaccharides.

The oats and wheat covered with cheesecloth grew slightly taller than the uncovered. In two of the four seasons there was some lodging in the general fields but none in the vicinity of the cages. In all four years, however, the covered grain lodged. Fig. 3 shows the lodging of wheat when shaded in this manner.

Under field conditions reduced light around the lower part of the culms may result from such cultural practices as thick seeding or the use of small seeds. In 1924 samples were gathered from oats seeded at the rates of 4, 14, and 24 pecks per acre, and from wheat sown at the rates of 3 and 10 pecks per acre. The analytical results, given in

table XII, do not show any material difference in the percentage composition of dry matter and total carbohydrates due to rate of seeding. However, on account of the reduced size in diameter of the

TABLE X

EFFECT OF SHADE ON COMPOSITION OF OAT AND WHEAT CULMS, 1923

MATERIAL	PERCENTAGE			
	OATS		WHEAT	
	Unshaded	Shaded	Unshaded	Shaded
Moisture.....	79.810	84.090	61.500	65.600
Dry matter.....	20.190	15.910	38.500	34.400
Nitrate nitrogen.....	0.034	0.027	0.020	0.002
Total nitrogen.....	0.159	0.172	0.118	0.129
Free reducing sugars.....	2.408	1.279	1.786	2.799
Disaccharides.....	0.819	0.508	8.068	0.840
Polysaccharides.....	3.596	2.621	7.078	6.353
Cellulose.....	2.606	2.156	4.275	4.850
Lignin.....	6.125	4.983	11.418	10.806
Total carbohydrates.....	15.554	11.547	33.525	25.648

TABLE XI

EFFECT OF SHADE ON COMPOSITION OF OAT AND WHEAT CULMS, 1924

MATERIAL	PERCENTAGE			
	OATS		WHEAT	
	Unshaded	Shaded	Unshaded	Shaded
Moisture.....	77.230	84.670	75.830	78.860
Dry matter.....	22.770	15.330	24.170	21.140
Nitrate nitrogen.....	0.004	0.002	0.001	0.002
Total nitrogen.....	0.103	0.059	0.060	0.067
Free reducing sugars.....	1.830	0.494	2.497	2.280
Disaccharides.....	0.378	0.000	7.086	1.902
Polysaccharides.....	4.283	2.969	4.551	3.989
Cellulose.....	3.412	2.899	2.932	2.980
Lignin.....	9.565	6.235	6.758	6.586
Total carbohydrates.....	19.468	12.597	23.824	17.737

culms, there was a material reduction in the quantity of all the constituents per given length of culm, as shown in table XIII.

Similar results were obtained from the use of large and small oat seeds. In a field plot test with oats, seeds of different grades were

used. The difference in size of kernels is such as can be obtained by passing the seeds repeatedly through a good fanning mill. In 1924, after the oats were cleaned, the calculated number of seeds per

TABLE XII

EFFECT OF RATE OF SEEDING ON COMPOSITION OF OAT AND WHEAT CULMS, 1924

MATERIAL	PERCENTAGE				
	OATS IN PECKS PER ACRE			WHEAT IN PECKS PER ACRE	
	4	14	24	3	10
Moisture.....	80.360	79.500	80.950	73.460	72.770
Dry matter.....	19.640	20.500	19.050	26.540	27.230
Nitrate nitrogen.....	0.049	0.003	0.005	0.004	0.003
Total nitrogen.....	0.069	0.062	0.067	0.126	0.104
Free reducing sugars.....	1.829	1.670	1.147	8.480	6.185
Disaccharides.....	0.261	0.653	0.944	0.073	0.000
Polysaccharides.....	3.177	3.353	3.300	5.516	5.114
Cellulose.....	2.925	3.497	3.181	3.104	3.974
Lignin.....	8.636	8.686	7.908	8.434	9.074
Total carbohydrates.....	16.828	17.859	16.480	25.607	24.347

TABLE XIII

EFFECT OF RATE OF SEEDING ON COMPOSITION PER UNIT LENGTH OF CULM

CONSTITUENTS PER CENTIMETER OF CULM (MG.)

MATERIAL	OATS IN PECKS PER ACRE			WHEAT IN PECKS PER ACRE	
	4	14	24	3	10
Moisture.....	99.209	67.948	37.476	56.725	30.601
Dry matter.....	24.247	17.521	8.819	20.494	11.451
Total nitrogen.....	0.085	0.053	0.031	0.097	0.044
Free reducing sugars.....	2.258	1.427	0.531	6.548	2.601
Disaccharides.....	0.322	0.558	0.437	0.056	0.000
Polysaccharides.....	3.922	2.866	1.528	4.259	2.151
Cellulose.....	3.611	2.089	1.473	2.397	1.671
Lignin.....	10.661	7.424	3.661	6.513	3.816
Total carbohydrates.....	20.775	15.264	7.629	19.773	10.238
No. culms per 100 gm...	18	30	60	35	58
Av. length of culms cm...	45	39	36	37	41
Total length of culms....	810	1170	2160	1295	2378

bushel was 779,520 for the large and 1,835,520 for the small. The two classes of seeds were sown at three different rates: 4, 14, and 24 pecks per acre. The composition of the culms, given in table XIV,

does not show any material difference in the percentage of dry matter and total carbohydrates. The culms produced from the small seeds,

TABLE XIV

EFFECT OF SIZE OF SEED ON COMPOSITION OF OAT CULMS, 1924;  
LARGE VERSUS SMALL SEED OATS

MATERIAL	PERCENTAGE					
	4-PECK RATE		14-PECK RATE		24-PECK RATE	
	Large	Small	Large	Small	Large	Small
Moisture .....	81.760	79.560	78.260	77.450	78.740	78.450
Dry matter .....	18.240	20.440	21.740	22.550	21.260	21.550
Nitrate nitrogen .....	0.002	0.003	0.002	0.002	0.001	0.002
Total nitrogen .....	0.068	0.062	0.067	0.056	0.073	0.056
Free reducing sugars .....	1.510	1.786	1.481	1.554	1.525	1.771
Disaccharides .....	0.639	0.886	1.829	1.525	1.670	1.074
Polysaccharides .....	3.114	3.382	3.771	3.924	4.019	3.584
Cellulose .....	2.920	2.965	3.158	3.160	3.018	3.382
Lignin .....	7.796	8.554	8.642	8.648	8.512	9.340
Total carbohydrates .....	15.979	17.573	18.881	18.811	18.744	19.151

TABLE XV

EFFECT OF SIZE OF SEED ON COMPOSITION PER UNIT LENGTH OF OAT CULM

CONSTITUENTS PER CENTIMETER OF CULM IN MG.

MATERIAL	4-PECK RATE		14-PECK RATE		24-PECK RATE	
	Large	Small	Large	Small	Large	Small
Moisture .....	100.073	61.818	63.110	31.716	38.001	27.938
Dry matter .....	22.325	15.882	17.532	9.234	10.260	7.675
Total nitrogen .....	0.082	0.048	0.054	0.023	0.035	0.020
Free reducing sugars .....	1.848	1.388	1.194	0.636	0.736	0.631
Disaccharides .....	0.782	0.688	1.475	0.624	0.806	0.382
Polysaccharides .....	3.812	2.628	3.041	1.607	1.939	1.276
Cellulose .....	3.574	2.304	2.547	1.294	1.457	1.204
Lignin .....	9.542	6.646	6.696	3.541	4.108	3.330
Total carbohydrates .....	19.558	13.654	15.226	7.703	9.046	6.820
No. culms per 100 gm. ....	19	33	31	74	56	78
Av. length of culms cm. ....	43	39	40	33	37	36
Total length of culms .....	817	1287	1240	2442	2072	2808

however, were materially smaller in diameter, and on account of this fact the constituents per given length of culm, as shown in table XV, were significantly reduced.

**DIAMETER AND BREAKING STRENGTH OF CULMS.**—Either thick seeding or the use of small seeds gives an increase in the stand of plants, and this results in a decrease in the diameter and hence in the breaking strength of culms. The average diameter and breaking strength of oats sown at the rates of 4 and 14 pecks and of wheat sown at the rates of 3 and 10 pecks per acre, and of oats grown from large and small seed are shown in table XVI. The weight of 100 sections, each taken from a separate culm, is also shown for each of these classes of seed.

From table XVI it is evident that a thickening of the stand affected adversely in all cases the strength of the culms. It decreased

TABLE XVI  
EFFECT OF THICK STAND ON DEVELOPMENT OF CULMS

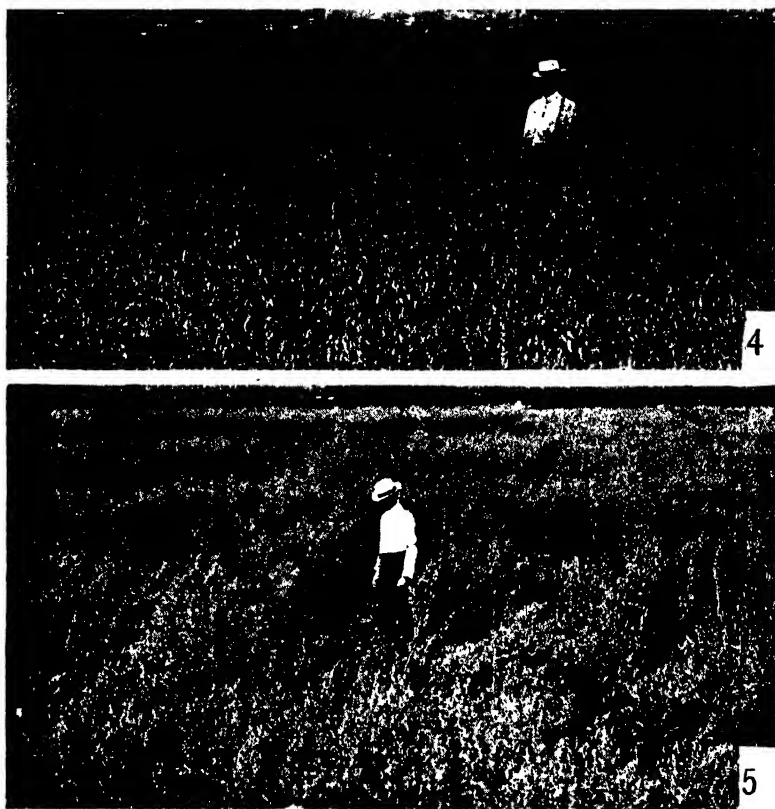
MATERIAL	OATS			WHEAT		
	Diameter (mm.)	Breaking strength (gm.)	Weight of 100 sections 8 cm. long (gm.)	Diameter (mm.)	Breaking strength (gm.)	Weight of 100 sections 8 cm. long (gm.)
Thin seeding .....	5.24	1329	134.5	3.39	573	91.0 ✓
Thick seeding .....	4.09	712	78.2	2.83	370	63.1 ✓
Large seed .....	4.61	1115	100.0	.....	.....	.....
Small seed .....	3.60	690	78.0	.....	.....	.....

the diameter and breaking strength and reduced the density, as indicated by the weight of a group of sections of definite number and length.

The culms were gathered soon after the grain had reached the full heading stage, and the weights and measurements were made on the fresh material. In the oats the diameter and breaking strength were determined at the fourth node below the head, the diameter being measured just below and the breaking strength on the node. In the wheat the determinations were made in the same way, but at the third node below the head.

In an annual rate-of-seeding field test with oats it has often been observed that the degree of lodging is correlated with the rate of seeding; the heavier the rate the greater the amount of lodging. In this test the rates of seeding vary from one another by one peck, and

they extend from 4 to 14 pecks inclusive. In lodging years the falling down usually begins at the 5- or 6-peck rate, and increases as the rate of seeding increases. Figs. 4 and 5 show the erect culms from seeding oats at 4 pecks per acre and the lodging produced when 14 pecks per acre are used.



FIGS. 4, 5.—Oats: fig. 4, four pecks per acre; fig. 5, fourteen pecks per acre

**TEMPERATURE.**—In order to determine whether there is any relationship between temperature and lodging, an area each of oats and wheat approximately 6 by 6 feet was inclosed with window glass. Of course the glass changed the quality of light, and in order to overcome this source of error a second area of both oats and wheat,

each approximately 12 by 12 feet, was covered with window glass, the sides being left open so as not to affect appreciably the temperature. The covering was raised from time to time as the oats and wheat increased in height. It was thought that this arrangement would result in light of the same quality in both areas, and that any difference in composition of the plants grown under the two conditions might therefore be attributed, in large measure at least, to the higher temperature in the inclosure. Of course there was some difference in humidity, but this was not great. The rate of evaporation, as shown by atmometers, was somewhat greater inside than outside the inclosures. No artificial heat was applied, and hence the amount of increase within was dependent entirely on the outside temperature. On hot sunshiny days the rise was higher than on cool cloudy days. On warm days the maximum increase ranged from 8° to 10° F. The temperature under the glass cover, even on the warmest days, rarely exceeded that outside by more than one or two degrees.

Small areas of oats were covered with these two kinds of frames June 26, 1924, when the plants were about 18 inches tall and growing vigorously. In the inclosure the oats lodged badly on July 10, at which time they were well in head. At the same time there was slight indication of lodging under the glass cover, but the oats did not go down there until considerably later, and never as badly as in the inclosure. On July 22 a sample was taken from the inclosure, one from under the glass cover, and one from the open. The oats in the inclosure were preceptibly taller than were those under the glass cover, and those under the cover were somewhat taller than were those growing in the open.

Areas of wheat were covered with similar frames of glass April 23, 1924, at which time the young wheat was approximately 4 inches tall. The wheat in the glass inclosure headed out about two days in advance of that under the glass cover, and the latter preceded the general field by approximately one day. The wheat in the inclosure showed first heads June 7. It lodged badly June 14, at which time the wheat under the glass cover also showed a slight tendency to lodge. Later the lodging of the wheat under the glass cover became more pronounced, but it never equaled that in the inclosure. All of the wheat in the general field, except that in a low and therefore

more fertile portion, remained erect throughout the season. The wheat in the inclosure was appreciably taller than was that under the glass or in the open field. Samples from the glass inclosure, from under the cover, and from the open were taken for analysis June 19. In taking a sample for analysis it was necessary to select some culms that were not entirely erect, for nearly all of them had partially or completely fallen. The results of the analyses of the oat and wheat culms are given in table XVII.

TABLE XVII  
EFFECT OF TEMPERATURE ON PERCENTAGE COMPOSITION OF  
OAT AND WHEAT CULMS, 1924

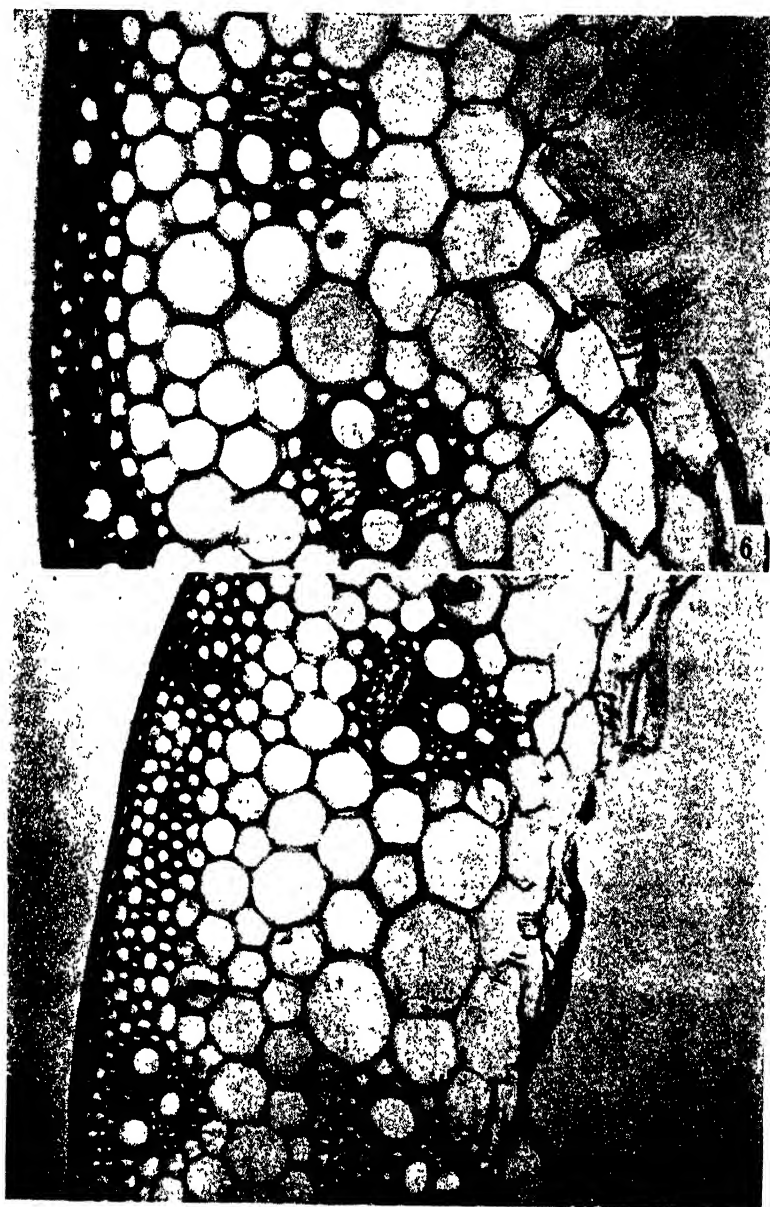
MATERIAL	OATS			WHEAT		
	Open	PROTECTED BY GLASS		Open	PROTECTED BY GLASS	
		Cover	Inclosure		Cover	Inclosure
Moisture . . . . .	77.230	79.860	82.800	68.440	73.900	78.070
Dry matter . . . . .	22.770	20.140	17.200	31.560	26.100	21.930
Nitrate nitrogen . . . . .	0.002	0.002	0.002	0.001	0.001	0.002
Total nitrogen . . . . .	0.103	0.114	0.097	0.051	0.065	0.059
Free reducing sugars . . . . .	1.830	0.769	0.610	1.554	1.234	1.016
Disaccharides . . . . .	0.378	0.450	0.319	8.959	5.735	2.062
Polysaccharides . . . . .	4.283	3.398	3.035	6.194	4.943	4.849
Cellulose . . . . .	3.412	3.386	3.202	4.292	4.301	4.239
Lignin . . . . .	9.565	8.498	7.115	8.308	8.827	7.801
Total carbohydrates . . . . .	19.468	16.501	14.281	29.307	25.040	19.967

From table XVII it may be seen that the plants grown in the inclosure contained a higher content of moisture, a lower content of dry matter, and a lower content of all the carbohydrate compounds. The greater contrast in the composition of wheat than in oats is probably due to the fact that the former was covered at a much earlier stage of development than was the latter.

#### ANATOMICAL

Cross-sections made on the stems show that the different environmental conditions affect materially the development of the various kinds of tissue contained in the culms. The effect on the structure of wheat culms of moderate differences in fertility, such as may be found in any field between hilltop and valley, is very





FIGS. 6, 7.—Oat culms: fig. 6, unshaded; fig. 7, shaded

marked. The thickness of the culm wall grown on high ground is usually about one-third greater, and the hypodermal zone is much more completely lignified, than in culms grown on more fertile low ground.

The effect of shade on the development of oat culms is shown in figs. 6 and 7. In the unshaded oats the width of the zone of parenchyma tissue is greater than in the shaded. Although the width of the hypodermal zones is about equal in the two, the quantity of lignified tissue is greater in the unshaded. In the shaded wheat culms the zones of parenchyma and hypodermal tissues are both somewhat greater than in the unshaded stems. More lignified tissue also is found in the unshaded culm. These differences are illustrated in figs. 8 and 9.

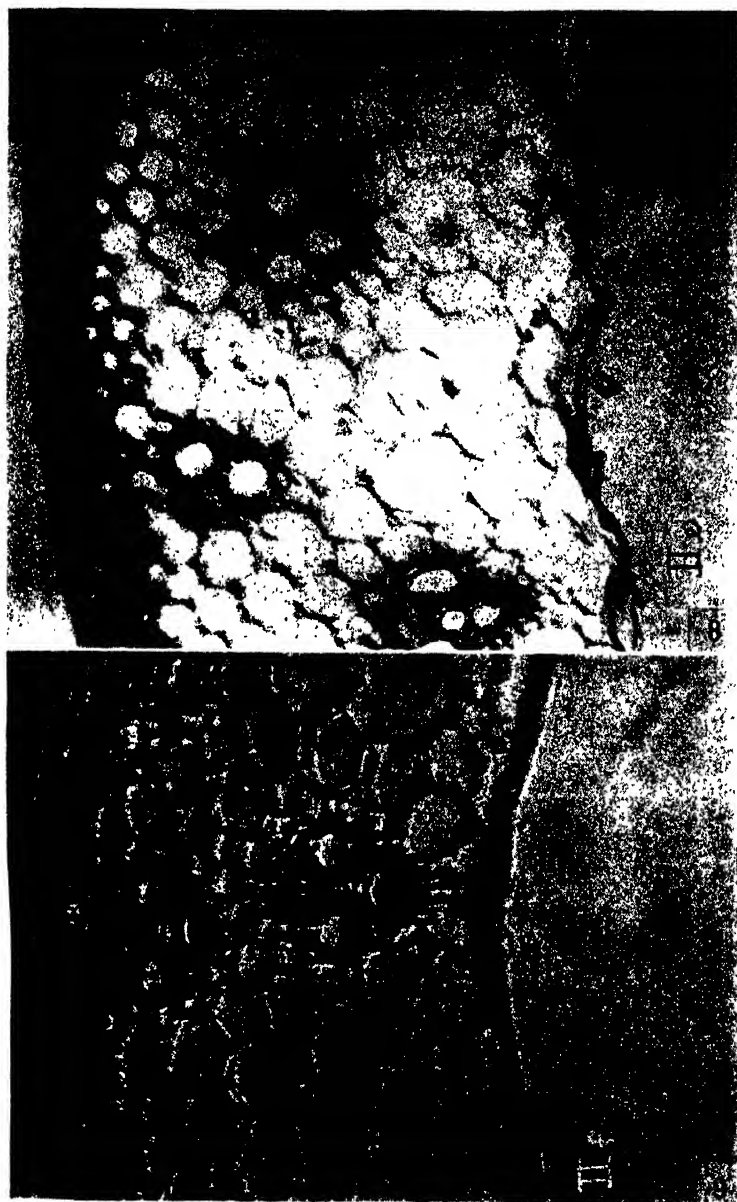
Rate of seeding also affects the structure of the culm. Stems taken from oat seedlings made at the rates of 4 and 14 pecks per acre are shown in figs. 10 and 11 respectively. Although the total thickness of walls in the two seedlings was practically equal, the width of the hypodermal zone is much wider in the thin seeding.

The difference in thickness of culm wall of wheat sown at the rates of 3 and 10 pecks per acre is shown in figs. 12 and 13 respectively. The total width of the culm wall was greater in the thin than in the thick seeding. Although the zone of hypodermal tissue was somewhat narrower, the cells were more completely lignified and compact.

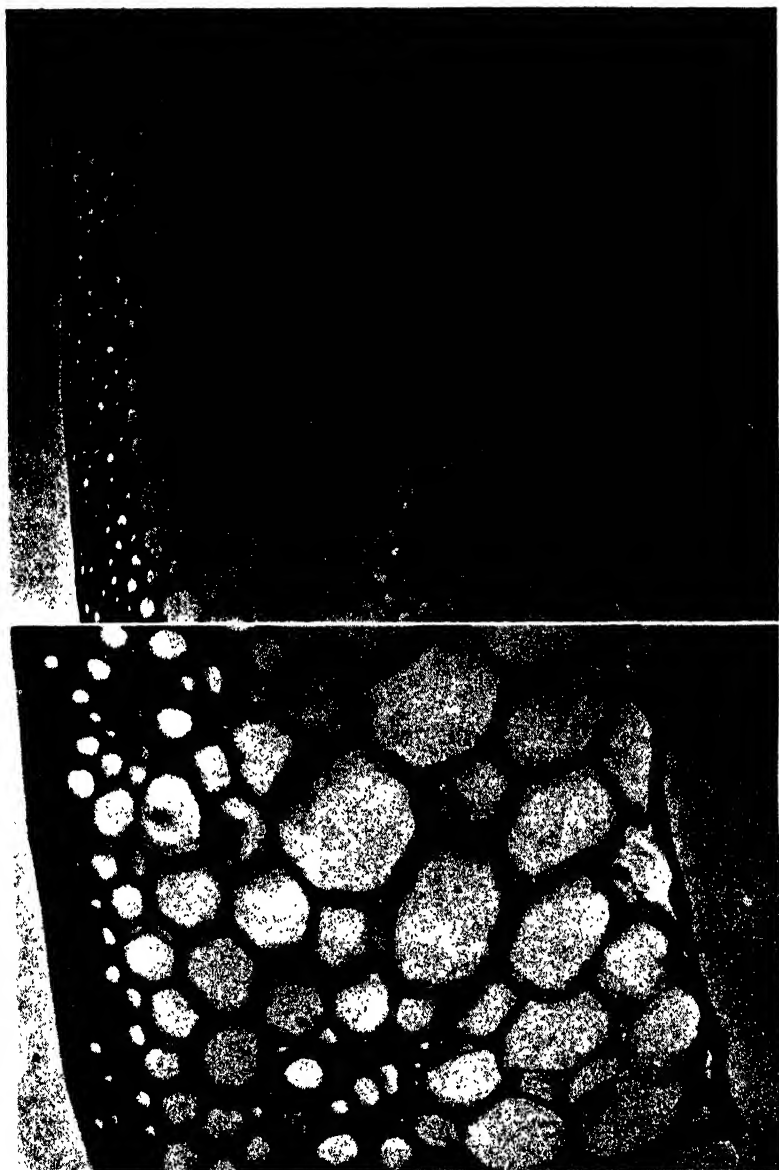
The effect of the use of large and small seed on the structure of oat culms was observed, and it was found that the total width of the culm was greater from the use of the large seed. The width of the hypodermal zone and the amount of lignification were also greater in the culm grown from the large seed.

### Discussion

In field practice lodging is much more prevalent on rich than on poor land. Analyses of samples taken in 1924 from rich low land and from poor high land showed that the culms of oats and wheat taken from the former contained a higher moisture and a correspondingly lower dry matter content than did those grown on the latter. The plants with the lower dry matter content lodged.

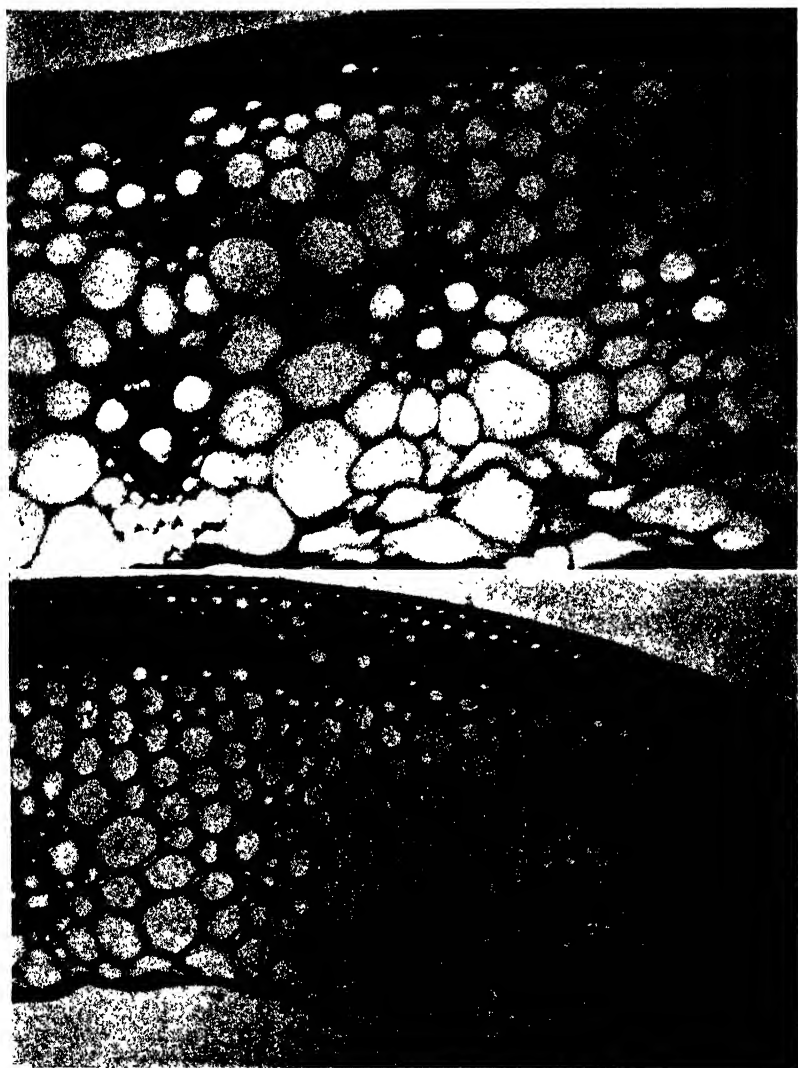


FIGS. 8, 9.—Wheat culms: fig. 8, unshaded; fig. 9, shaded



FIGS. 10, 11.—Oats: fig. 10, four pecks per acre; fig. 11, fourteen pecks per acre

In each of the three sand-soil-manure tests, analyses of the culms of oats and wheat showed that those grown in the manure contained a higher moisture and a correspondingly lower dry matter content



FIGS. 12, 13.—Wheat: fig. 12, three pecks per acre; fig. 13, ten pecks per acre

than did those grown in either the soil or the sand. The plants with the lower dry matter content lodged.

In the shading tests analyses of the culms of oats and wheat showed that those grown under the cheesecloth contained a higher moisture and a correspondingly lower dry matter content than did those grown in the open. The plants with the lower dry matter content lodged.

In the temperature tests analyses of the culms of oats and wheat showed that those grown in the inclosure contained a higher moisture and a correspondingly lower dry matter content than did those grown either under the glass cover or in the open. The plants with the lower dry matter content lodged.

From these experiments, therefore, the conclusion would seem to be warranted that lodging in oats and wheat results from high moisture and correspondingly low dry matter content of the culms. This is in accord with the findings of RIVERA (29), *op. cit.* with wheat, concluded that lodging was due to high salt from hypertonic tissue. The factors of which this situation is the result, says, the "true cause" of lodging. Results from the interaction of

The relatively low dry matter hypertonicity not only tends to be due to a relative elongation of the culms, but through a stimulation of the elongation processes, it increases the thickness of stand and hence results in a greater degree of shading around the base of the plants. In warm rainy cloudy seasons, therefore, the conditions are most favorable for the development of lodging on rich soils.

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## VASCULAR ANATOMY OF RANALIAN FLOWERS

### II. RANUNCULACEAE (CONTINUED), MENISPERMACEAE, CALYCANTHACEAE, ANNONACEAE

GEO. HUME SMITH

(WITH SIXTEEN FIGURES)

The vascular anatomy of the typical flowers of several genera of the Ranunculaceae has been studied by the writer, and the conclusion reached that the family is primitive (17). This inquiry into the fundamental nature of the Ranalian flower has been extended, in the present paper, to include certain unusual flowers of the genera *Ranunculus*, *Caltha*, *Delphinium*, and to include the families Menispermaceae, Calycanthaceae, and Annonaceae. Each of these groups shows additional evidence as to the nature of the Ranalian flowers.

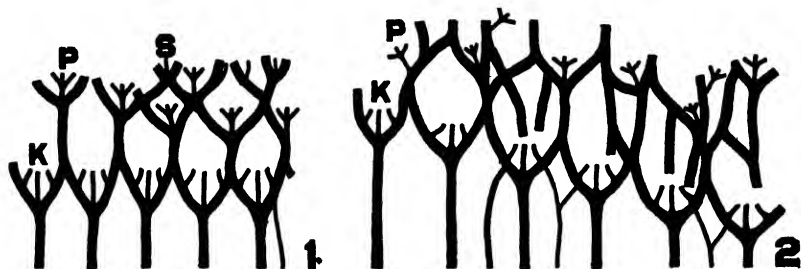
#### Ranunculaceae

In continuing the study of the Ranunculaceae, four variations from the usual floral organization will be considered. The first group includes those unusual flowers of *Ranunculus* in which the number of petals is more than five. For instance, during the past few seasons the writer has collected flowers of *R. abortivus* L., *R. acris* L., *R. recurvatus* Poir., and *R. septentrionalis* Poir. having more than five petals. Of these four species *R. septentrionalis* is especially prolific in the production of flowers with six, seven, or even eight petals, and in *R. acris* the variations are frequent. The second group includes the horticultural forms of *Ranunculus*. In these forms the flowers are completely "double"; that is, "the stamens and the carpels are completely replaced by petals." The third group considers a horticultural form of *Caltha palustris* in which "the stamens and the carpels are completely replaced by petals"; that is, the flower is completely double. The fourth group discusses horticultural forms of *Delphinium* in which the lowest stamens are replaced by petals.

1. FLOWERS OF *RANUNCULUS* WITH MORE THAN FIVE PETALS.—  
In the genus *Ranunculus* the flowers usually have five petals, but

individual flowers having six, seven, eight, or nine petals are not infrequent.

The vascular skeleton of a flower with more than five petals is represented by fig. 1. This figure is an actual tracing of the course of the vascular strands of a flower of *R. septentrionalis* with six petals, and can be taken as typical of any flower of *Ranunculus* having more than five petals. The axis is represented as split open and flattened out in one plane. The broader lines represent axial strands; the narrower lines represent the departing traces. The figure maps completely all strands of the sepal and petal region, and in addition



FIGS. 1, 2.—Fig. 1, *Ranunculus septentrionalis*: vascular system of axis of sepal (K), petal (P), and lower stamen (S) region split open and spread out in one plane; note presence of six distinct petals (P). Fig. 2, *Caltha palustris*: vascular system of completely double flower as if split open and spread out in one plane, showing sepal (K) and lower petal (P) region.

shows the first four stamens. If the higher levels were shown they would be the same as those pictured for the flower with the usual number of petals. The vascular skeleton of flowers having seven, eight, or nine petals differs from the flower portrayed in fig. 1 only in that a spiral consisting of a correspondingly greater number of petal traces exists.

The anatomical condition just described for these unusual flowers of *Ranunculus* suggests additional arguments to substantiate the contention that the petals and stamens of *Ranunculus* are closely related structures. For example, in the five-petal flowers of *Ranunculus* both petal and stamen are fundamentally single-trace appendages. In the flower with more than five petals, the petals and stamens are not only single-trace appendages, but also are arranged

in one continuous unbroken spiral series. They show, therefore, a striking unity of form and arrangement. Again, any suggestion that the additional petals are merely lobes of a fundamental five-petal condition, due to a complete splitting of one or more of the five petals, is not possible for several reasons. For instance, the trace to each petal departs from a different level and from a different point on the axial cylinder, and not in a progressive spiral succession and equally distant from each other. Another proof that each additional petal is a unit is deduced from the fact that each petal has a nectariferous scale at its base, regardless of whether the flower has five petals or more. Also it is to be noted that, whether the number of petals is five or more, each petal is supplied in identically the same fashion as every other petal; that is, each petal receives a single trace which arises directly from the axial vascular cylinder itself and which branches into three veins just as it enters the petal.

In conclusion, therefore, flowers of *Ranunculus* having more than five petals differ from the more usual five-petal flowers only in that the number of petals is variable (indefinite) and the insertion of the petals is spiral. As these two features, variability in the number of appendages and spiral arrangement of appendages, are indicative of a primitive floral organization, these unusual flowers represent a more primitive, a more *Caltha*-like organization than the usual five-petal flowers. Furthermore, a very close morphological relationship between petal and stamen is revealed by a study of the unusual flowers of *Ranunculus*, due to the fact that they are not only both single-trace appendages, but also are inserted in a continuous uninterrupted spiral sequence.

2. DOUBLE FLOWERS OF *RANUNCULUS*.—Horticulturists have succeeded in obtaining strains of *Ranunculus* in which the "stamens and the carpels are completely replaced by additional petals." Of these horticultural varieties, flowers of *R. septentrionalis* and *R. acris* have been studied. A reconstruction of the vascular skeleton of this type of flower displays one very important difference from a flower having several petals; namely, every trace above the sepal level, a total of sixty or more, instead of the first six to nine traces, partakes of the characteristics of petal traces; that is, each trace divides into three main veins as it passes through the zone transi-

tional between axis and appendage. This "double flower," therefore, either represents one in which both "stamens and carpels have been changed into petals," or one in which the stamens have been transformed into petals and the terminal carpellary region has been suppressed.

Four facts point to the conclusion that the carpels have been completely suppressed and all of the petals of these double flowers of *Ranunculus* are the homologues of stamens. The first is that each of the sixty or more appendages above the sepal level has a nectariferous scale at its base, just as each of the five petals of the ordinary flower has a nectariferous scale at the base. The second is that all appendages above the sepal level are supplied with the petal type of trace, namely, a single trace which branches into three veins. The third is that the total number of the petals of a double flower is approximately equal to the total number of petals and stamens of the usual flower of *Ranunculus*, rather than equal to the total number of petals, stamens, and carpels of the usual flower. The fourth is that, in the double flower, the series of departing traces is unbroken from the level at which the first petal trace arises until the axial vascular strands fade away as the last petal trace departs; whereas in the flower with five petals, and also in the flower with six to nine petals, a distinct interval exists between the point of departure of the last stamen trace and the first carpellary trace. Whether the axial interval between stamen and carpel in these flowers represents a single elongated internode or a region from which appendages have been suppressed does not enter into this question. The mere fact that the interval is not present in the double flower suggests that the petals of these flowers form one continuous unit, and the carpellary region has been suppressed.

In conclusion, therefore, the double flower of *Ranunculus* is one in which two modifications have taken place: (1) all of the stamens, not the lowest nine or less, have become transformed into petals; (2) the entire carpellary region has been suppressed.

3. DOUBLE FLOWERS OF *CALTHA PALUSTRIS*.—*Caltha palustris* normally has no petals, but a horticultural form exists in which the "stamens and the carpels are replaced by expanded petal-like appendages." In color and shape these appendages are similar to se-

pals, but they are decidedly smaller. The outermost are about one-fourth the size of the sepals, and they gradually diminish in size until the innermost are very small. Even the innermost are not tipped with pollen sacs, and therefore no transition from petal to stamen can be traced, as is possible, for example, in *Castalia*.

The basal portion of the vascular skeleton of a "double flower" is portrayed in fig. 2. As in the normal flower of *Caltha*, traces depart from the axial vascular cylinder, so that each sepal is supplied with three traces. Then, continuing the spiral, traces depart to the petals, each of which receives one trace. Just as this trace passes out of the receptacle into the petal it branches into three prongs, forming the three principal veins of the petal. As the apex of the receptacle is approached, the axial strands rapidly diminish in size. For instance, a cross-section of the axis at 1000  $\mu$  from the apex reveals that each axial strand contains eight or more lignified elements. At 600  $\mu$  the strands have decreased to about a third of their former dimensions, and the lignified elements have decreased to two or three in each strand. At 400  $\mu$  the strands are entirely devoid of lignified elements, and are scarcely distinguishable from the surrounding parenchyma. A section at 200  $\mu$  from the apex discloses no circle of bundles. The tissue is homogeneous parenchyma. Twenty or more petals arise above this point and hence are without a vascular supply. This condition is directly the reverse of the wild form, in which the remains of a vascular supply to completely suppressed terminal carpels exist; whereas in the double flower the axial vascular supply gives out before the uppermost appendages (homologues of stamens) are supplied, and all evidence of a carpellary region has vanished.

In comparing the vascular skeleton of the double flower of *Caltha* with the uncultivated apetalous form, three striking similarities between the two types appear. The number of sepals and stamens of the one approximately equals the number of sepals and petals of the other. This similarity and the two following, therefore, both argue for the homology of the petals of the double flower of *Caltha* and the stamens of the apetalous uncultivated flower. The second similarity between the two flowers is that the appendages in both cases are inserted in a spiral. The third similarity between the two

flower types, and incidentally a third argument for the similarity of petals and stamens, is that each sepal in both types of flower receives three traces, and each petal of the one flower and each stamen of the other flower receive a single trace.

On the other hand, the vascular skeleton of the double flower of *Caltha* differs from that of the common apetalous flower in two respects. In the first place, the double flower has no equivalent of the carpellate region of the normal apetalous flower. Three reasons already have been stated to show that the petals of the double flower are the equivalent of the stamens of the apetalous flower, and certain additional facts can be presented to substantiate this assertion. It was obvious in the study of the normal apetalous flower (17) that the more terminal carpels had disappeared, and it is obvious in the double flower that the axial bundles rapidly diminish in size as the apex of the receptacle is approached, and thus indicate that a considerable portion of the receptacle is suppressed. In the second place, the double flower of *Caltha* differs from the apetalous flower in that each trace passing to an appendage above the sepal level branches into three main veins, just as it passes through the parenchyma transitional at the point of union of the petal and receptacle, whereas in the wild apetalous form the conditions are different. The trace to the stamen passes as a single unbranched unit the entire length of the appendage, and at the higher levels the traces depart in groups of three as dorsal and ventral traces to the individual carpel.

In conclusion, therefore, the vascular skeleton of the horticultural form of *Caltha palustris* is fundamentally that of the wild apetalous form, from which it differs in but two respects: (1) the stamens have become transformed into expanded petal-like structures similar to the petals of *Ranunculus*; (2) not only has vascular supply to terminal suppressed carpels disappeared, but even the entire carpellate region and also the vascular supply to the uppermost petals (homologues of stamens) has disappeared.

4. INCOMPLETELY DOUBLE FLOWERS OF DELPHINIUM.—The vascular structure of the incompletely double flower of *Delphinium* cannot be understood until the structure of the normal flower has been described. In this flower (5 sepals, 4 petals) the three traces to each

sepal arise from separate and distinct points on the axial vascular cylinder, and three separate and distinct gaps in the axial vascular cylinder are formed. Above this level the axial strands subdivide and recombine, so that the cylinder becomes the usual network studded with departing traces. The traces of the petal-stamen region emerge from this network in a suggestively spiral fashion. Each trace that departs from the axial cylinder within this region becomes the entire supply to an appendage. The first four appendages above the sepal on the adaxial side are the petals, whereas the appendages of the same turn of the spiral on the abaxial side are stamens; that is, petal and stamen form one continuous series. As in *Ranunculus*, *Aquilegia*, and *Callia* (double flower), the trace to a petal branches into three parts as it passes through the parenchyma transitional between petal and receptacle, whereas the stamen trace is entirely unbranched (fig. 1). After the stamens are supplied the axial strands become three; each of these strands becomes the supply to a carpel. In the basal part of each carpel the strand branches into its component three traces, one dorsal and two ventral.

The vascular structure of the double flower differs from the usual flower in only one respect. Each of the twelve to fifteen appendages above the sepal level has not only the external appearance of petal, but also receives the typical petal type of vascular supply, namely, a single trace which branches into three veins as it enters the base of the petal; whereas in the usual flower only the first four appendages on the adaxial side have the appearance and vascular supply of petals. In such other important respects as the combined total of petals and stamens the two flowers are the same; consequently, following the type of argument used in the discussion of the double flowers of *Ranunculus*, it can be proved that the petals and stamens of *Delphinium* form one continuous unit and are fundamentally similar.

In conclusion, therefore, it can be said that the vascular anatomy of the double flower of *Delphinium* illustrates and substantiates all of the conclusions drawn from the vascular structure of the preceding three groups.

#### DISCUSSION

In reviewing the data presented by the unusual forms of the Ranunculaceae, additional evidence is brought forth as to the nature

of the petal. For instance, the vascular anatomy of these flowers shows that every petal is a single unit. The details of this argument were presented in the discussion of the flowers of *Ranunculus* having six or more petals.

The facts also demonstrate that the vascular supply to the petal displays an individuality of its own. In the ordinary flower of *Ranunculus*, *Aquilegia*, and *Delphinium* it was noted that every petal trace branches into three veins as it enters the base of the petal. In this study it is shown, for instance, that every flower of *Ranunculus septentrionalis*, whether the petals be five, several, or many, has this type of supply to each of its petals. The same can be said of *Delphinium*; and each of the sixty or more "petals" of *Caltha* are so supplied. The conclusion, therefore, is that this is a distinctive anatomical characteristic of petals whenever they occur in such members of the Ranunculaceae as *Ranunculus*, *Aquilegia*, *Delphinium*, and *Caltha*.

The question now arises as to whether the petals of these genera are distinct morphological units, morphological units closely related to the sepals, or morphological units of staminal affinity. The petals cannot be sepals, because of the difference in method of vascular supply. This difference is most clearly illustrated in the double flower of *Caltha*, *Ranunculus*, and *Delphinium*. Each sepal is supplied with three distinct traces, each petal with a single strand. If the single strand represented three laterally fused traces, some transitional stages certainly would have been observed in some of the flowers examined, but such have not been found. Thus no similarity to sepals exists. On the other hand, each of the forms studied gives evidence of the staminal affinity of the petals. The first is that the vascular supply to a petal, as that of a stamen, arises as a single trace. Again the axial vascular skeleton of the petal region of the double flower of *Caltha* has the exact ground plan of the stamen region of the single apetalous wild flower. Similarly, in *Delphinium* the stamen-petal region is a single unit. Also in *Ranunculus* the vascular skeleton of a flower having many petals presents the same plan as the petal-stamen region of the flower having six to nine petals, and this in turn is very similar to the flower having a whorl of five petals and an indefinite number of spirally arranged stamens. It seems obvious, therefore, that stamens and petals are closely related organs.



The evidence just presented to show the staminal affinities of petals also more closely links *Ranunculus* to *Caltha* than before. In the six-nine petal flower it is to be recalled that the insertion of the petals is spiral, not cyclic. It is also established that these petals are stamens; therefore the *Ranunculus* flower of several petals is in reality a flower composed of five whorled sepals, several spirally inserted modified stamens (petals), and an indefinite number of true stamens and carpels. It is also known that, in general, the indefinite number of parts has given rise to the definite number, and the spiral to the cyclic. If one pictures such changes as these in the organization of the calyx and the corolla of *Ranunculus*, and considers all stamens as unmodified, then *Ranunculus* is transformed into the more primitive *Caltha* type.

These forms also present evidence to show that the vascular tissue may disappear before the organ to be supplied. It will be recalled that in the double flower of *Caltha*, the vascular axial cylinder fades away before the uppermost and smallest petals (homologues of stamens) are supplied, whereas in the more typical apetalous form not only are all of the appendages supplied, but remnants of vascular supplies to apical and suppressed carpels exist.

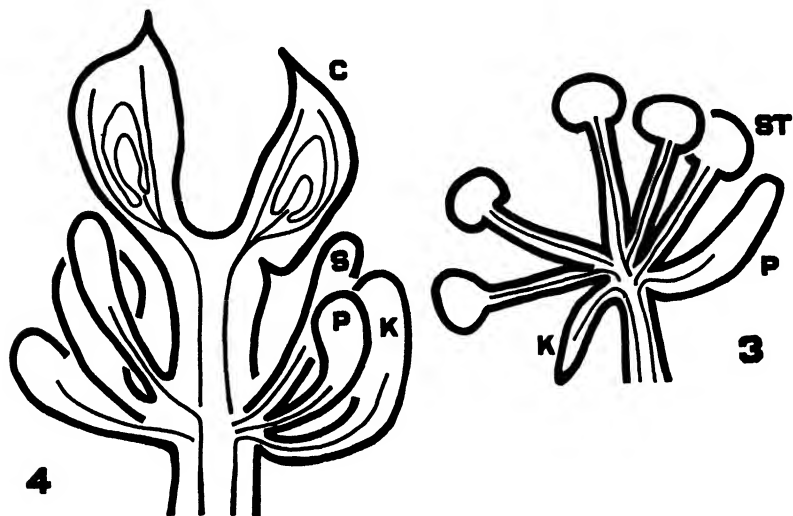
The unusual flower of the Ranunculaceae, therefore, whether partially or completely double, is built upon the same general plan as the normal flower; and when completely double, as in *Ranunculus* and *Caltha*, the carpellate region is totally suppressed. Furthermore, these unusual flowers show that all petals are modified stamens because of their single trace character, their formation of the continuous spiral with the stamens, their complete replacement of stamens of the double flowers in *Caltha* and *Ranunculus*, and the partial replacement in *Delphinium*.

### Menispermaceae

If *Menispermum canadense* L. is taken as typical of the Menispermaceae, then the flower of this family shows certain distinct variations in vascular anatomy from the Ranunculaceae. Because of these variations the vascular anatomy of the flower is of interest in determining the nature of the Ranalian flower.

ANATOMY OF FLOWER.—In the staminate flower of *M. canadense* (fig. 3) the pedicel usually contains five separate and distinct strands.

At the sepal level these divide radially. Of the strands which result from this radial division some remain axial, and others supply the four to eight sepals with one trace each. Immediately thereafter these axial strands redivide and supply the petals in a like manner; then the remaining axial strands divide into a number of traces equal to the number of stamens. Occasionally, however, stamens which appear to be perfectly normal contain two distinct traces throughout their entire length. No special significance can be attached to this



FIGS. 3, 4.—*Menispermum canadense*: median longitudinal section of staminate flower (3), and of pistillate flower (4); K, sepal; P, petal; ST, stamen; S, staminodium; C, pistil.

abnormality. The vascular framework of the staminate flower (fig. 3) thus displays a very marked resemblance to the staminate flower of *Thalictrum dioicum* of the Ranunculaceae.

In the fertile flower (fig. 4) the sepals, petals, and staminodia (non-functional stamens and usually six in number) are supplied in a manner similar to that for the corresponding appendages of the staminate flower. The axial cylinder then continues upward as a group of more or less distinct strands. This portion of the axis forms the stalk, upon the distal end of which are the two to four carpels. At this point the strands arrange themselves into as many groups

as carpels, and then each group enters a single carpel. Sometimes these groups appear to be composed of four distinct strands; at other times completely united into a single strand. Within the base of each carpel one strand proceeds to the stigma along the dorsal margin; two others toward the stigma along the ventral margin; and the fourth, which is located between the two ventral strands, directly to the ovule.

#### DISCUSSION

The carpels, as the anatomical evidence discloses, are of the typical three-trace type. They differ from the more primitive *Caltha* carpel, however, in that only one ovule is present; all vestiges of additional ovules have been lost. A second difference from the Ranunculaceae is that the strand to the ovule frequently becomes a separate entity before the carpel supply leaves the axial cylinder. This precocious splitting of the carpellary strand, however, is of no particular significance.

The stamens are normally single-trace appendages. Thus their origin still remains to be solved. The literature of floral vascular anatomy (1-20) consistently reports stamens to be single-trace appendages.

The staminodia of the fertile flower are probably the homologues of stamens for several reasons. The evidence is not direct, but several circumstantial facts point in this direction. The sepals and petals of the fertile flower correspond to similar appendages of the staminate flower; hence the staminodia appear to be an additional group of appendages. The stamen-like external appearance of these appendages is another possible indication of a stamen affinity. Likewise the single trace condition is a stamen characteristic, but cannot absolutely be diagnostic because, as is discussed in the following paragraph, petals and sepals are likewise single-trace appendages, but probably of a different origin. Finally, the appreciable axial interval between the point of attachment of the staminodia to the receptacle and that of the carpels suggests that intervening appendages (additional stamens) have completely disappeared. The origin and development of such a condition are easy to picture, when one recalls the conditions of *Aquilegia canadensis* (17). In this flower the upper-

most whorls of stamens, although supplied with vigorous traces, are small, chaffy, and have undeveloped anthers. They are evidently on the way to complete atrophy, and the fertile flower of *M. canadense* can be taken as a very advanced stage of this tendency. A somewhat intermediate step might be cited in *Ulmus*, in which BECHTEL (4) found that some of the stamens had disappeared but the traces to these stamens still existed.

The petals and sepals are also single-trace appendages, consequently two views as to the nature of the petal are possible: petals may be considered as non-functional stamens or they may be considered as sepals. Similarly two views are possible as to the nature of the sepals: they may represent a group of modified petals and thus represent a secondary modification of stamens, or they may be considered as modified foliage leaves. In the latter case they have been derived from a more primitive multitrace type of foliage leaf, either by a loss of the lateral traces or a fusion of the lateral traces to the median to form a single strand. Thus the fact that sepals, petals, and stamens are all single trace appendages, and are in one continuous series, obscures the affinity or lack of affinity of these appendages for each other.

It is also to be pointed out that *Menispermum canadense* presents evidence to show that imperfect flowers have been derived from perfect flowers. In the derivation of the flower having only stamens the chain of evidence is very clear. For example, in an earlier portion of this paper it was noted that in the wild form of *Caltha palustris*, vascular tissue passes toward apical and totally suppressed terminal carpels; and that in the horticultural form in which petals have replaced the stamens, not only have the carpels disappeared, but also the vascular conditions indicate that the stamen-bearing portion is fading away. Thus, similarly, it is possible to consider that the staminate flower of *Menispermum* is the result of the complete disappearance of the apical carpellary, and even possibly part of the staminal region. As regards the fertile flower, the presence of at least six stamen-like filaments, an appreciable interval, and then the carpels suggests that intervening stamens have disappeared. This can be asserted in spite of the fact that no vascular tissue passes

outwardly within this interval toward suppressed stamens, because it is entirely possible for both an organ and its vascular supplies to disappear simultaneously.

If *Menispermum canadense* is typical of the Menispermaceae, therefore, the vascular anatomy of the flower of this family is of the same fundamental design as that of the Ranunculaceae, and is a specialized offshoot from the more primitive generalized *Caltha* type. In this specialization the anatomical facts point toward two modifications: (1) the flowers have become differentiated into purely staminate flowers and into perfect flowers with the stamens non-functional, through the disappearance of both appendages and traces; (2) the sepals, petals, and stamens are similar, in that all are single-trace appendages. It has been impossible to determine whether this similarity does or does not bespeak a morphological affinity.

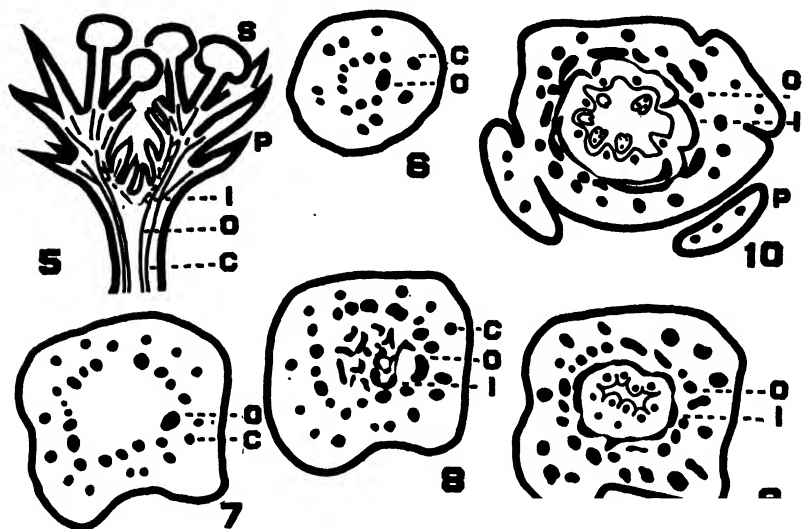
#### Calycanthaceae

In the Calycanthaceae the flower differs in form from the other families of the Ranales. Instead of the usual convex shape, the receptacle is deeply concave (fig. 5); in other words, the receptacle is inversely conical or cup-shaped. The carpels are placed upon the bottom and inner surface of the cup, the stamens upon the rim, and the perianth parts upon the outside. Because the shape of the flower is unusual, the distinctive features of the vascular anatomy are of particular interest in determining the relationship of this family to the other Ralian groups.

ANATOMY OF FLOWER.—The vascular framework of the flower of *Calycanthus floridus* L. is sufficiently typical to represent the entire family. The peduncle contains two concentric cylinders of vascular strands (figs. 5, 6). The cortical cylinder (C) is composed of about ten distinct strands, and the axial or outer cylinder (O) of about ten or fifteen strands. Upon reaching the region at which the first few perianth parts arise, the strands of the cortical system are increased slightly in number by radial divisions (fig. 7). These strands then pass upward and outward and enter the first few scattered perianth parts. Three or four adjacent strands enter a single perianth part. Thus the cortical cylinder of vascular tissue vanishes.

Next the course and the changing characteristics of the outer

cylinder of vascular strands will be followed from the peduncle upward to the rim. Slightly below the level at which the first group of strands from the cortical cylinder disappear into perianth parts, strands of the outer cylinder divide radially (fig. 7). Some of the resulting strands move outward and upward, and in turn enter higher perianth parts. The majority of the strands, however, remain in



FIGS. 5-10.—*Calycanthus floridus*: fig. 5, median longitudinal section of flower; figs. 6-10, cross-sections of flowers at successively higher levels; C, cortical cylinder of vascular tissue; I, inner cylinder of vascular tissue (inverted portion of outer cylinder); O, outer cylinder of vascular tissue (upright portion of axial cylinder); P, perianth part; S, stamen. Carpels seated on interior of cup.

alignment with the outer axial cylinder and continue to divide radially. At each of the divisions of an axial strand some of the segments become perianth traces, others remain as axial strands. Before traces have departed to many perianth parts the individuality of the strands of the outer cylinder gradually becomes obscured. This is due to the oblique passage of numerous elongated parenchymatous cells and spiral xylem elements from one upright axial strand to the next (fig. 10). As the rim of the receptacle is approached the outer cylinder again breaks up into distinctly individual strands. Some of these pass outward in groups of three or four to the uppermost perianth parts; others pass inward to the stamens which are

seated upon the inner rim of the receptacle. One trace enters each stamen. Thus a cross-section of a flower at the rim of the cup reveals groups of three or four vascular strands in various stages of outward upward passage to perianth parts, and also single strands passing inward, upward to stamens (fig. 5).

Such sections also reveal the interesting fact that a third zone of vascular tissue, inner cylinder (*I*), exists (fig. 10). The significance of this zone can be understood best by tracing its passage downward and inward from the rim of the cup toward the central basal portion of the receptacle. The strands of this cylinder can be distinguished from the strands of the outer cylinder because the xylem elements of the strands of the outer zone are in the normal centripetal direction, whereas the xylem elements of the strands of the inner zone are centrifugal. Furthermore, these bundles are very closely appressed to the vascular tissue of the outer zone for some distance downward. Frequently actual unions between the two cylinders occur (fig. 10). Only gradually do the strands of the inner zone converge toward the central basal portion of the receptacle (fig. 5). In the downward course of the strands of this cylinder, traces depart inwardly to the carpels. First the dorsal and then the two ventral traces depart to the carpel. The departure of the pairs of ventral traces accounts for the scalloped appearance of the inner cylinder of vascular tissue in fig. 10. The farther downward one follows the inner cylinder the less distinctly individual become the upright strands. This is due to the spreading out laterally of the spiral elements of one vertical strand toward the next. The cylinder thus becomes outlined as an interlacing mass of xylem elements. After the traces to the last carpels have departed and the receptacle has become a solid structure, the vascular elements of the inner cylinder become fewer in number, less lignified, and converge into a small group of elements in the basal central portion of the receptacle (fig. 8), and then finally disappear (fig. 7).

The course of the three traces which enter a carpel is very definite. One passes up the dorsal margin of the carpel, and the other two pass up the ventral margin as two distinct traces. If two ovules are present, each ventral trace gives rise to an ovular strand; if only one ovule appears, but one ventral trace puts forth an ovular strand.

## DISCUSSION

**CARPELS.**—The study of the anatomy of the carpels of *Calycanthus* reveals two interesting facts. The vascular structure is such that these carpels represent a condition intermediate between the three trace multiovulate carpel of *Caltha* or *Trollius* and the single strand (three traces united) uniovulate carpel of *Ranunculus* and *Hepatica*. It will be recalled that in the study of the Ranunculaceae (17) the multiovulate carpels of the *Caltha-Trollius* group displayed vascular conditions suggesting the gradual elimination of the apical ovules. *Calycanthus* would represent this tendency carried to the extent that all of the ovules but one or two have disappeared. The second interesting fact is that the ventral suture of a carpel is plainly visible, even after the ovules are well formed. In early stages of a carpel's development the two ventral margins can be found to be in close contact but not actually fused, except the upper third. The union of the ventral margins occurs rather late in the ontogeny of a carpel in this form.

**STAMENS.**—Each stamen receives a single trace. The literature of floral vascular anatomy (1-20) likewise records stamens as unique single-trace appendages.

**PERIANTH.**—The vascular structure of the perianth differs from that of the majority of the Ranunculaceae in two respects. The first difference is that the number of traces to a single appendage is either three or four, whereas among the Ranunculaceae the number is more typically three. However, *Clematis* frequently has four traces to a sepal. The second difference is that the perianth parts of *Calycanthus* range from the lower scattering bract-like structures through sepal-like structures to colored petal-like structures, all receiving the same type of vascular supply. Therefore the petals of the Calycanthaceae, if one calls the upper colored perianth parts petals, are distinctly sepaline in nature, whereas the petals of the Ranunculaceae are distinctly staminal in affinity.

**RECEPTACLE.**—The description of the receptacle of *Calycanthus* has revealed four interesting differences from the condition found in the Ranunculaceae. The first is that the receptacle is cup-shaped. The second is that the perianth parts are inserted on the outside of the cup, the stamens on the rim, and the carpels upon the inside.



The third is that the dorsal trace to a carpel departs from the vascular cylinder at a higher level than the two ventral traces. The fourth is that the upper portion of the receptacle contains two concentric systems of vascular tissue. Of these two systems the outer has the xylem tissue oriented toward the center, and the inner has the xylem tissue toward the outside. It is also to be noted that the two systems are not directly connected to form a continuous unit.

How could a floral primordium develop to fulfill these four conditions? If it is supposed that the rate of growth of the cells forming the apex of a normally convex floral primordium be checked (all portions of a meristematic region do not grow at the same rate), and the ring of meristematic tissue surrounding the very apex should continue to bulge up and grow, then the apex of the floral axis would become overshadowed by the bulging ring of tissue. As a result the floral appendages would spring from the outer and the inner surfaces of this overarching portion of the axis. The perianth parts would spring from the outer surface, the stamens from the rim, and the carpels would arise nearest the true apex, that is, from the interior of the cup. Consequently they would seem to be upside down. For this reason the dorsal trace does seem to arise at a higher level than the two ventral traces, that is, it arises nearer the rim, pseudo-apex of the cup. Again, because of the bulging of the meristem surrounding the apex, the axial vascular tissue would follow an inverted V-shaped course, and consequently the xylems of the upright and recurved portions would be facing each other. An actual union between the outer upright and inner recurved zones might be lacking and could be explained as follows. The close proximity of the two vascular zones at the point of sharp bending downward of the vascular tissue has resulted in frequent secondary unions, as mentioned in the description and noted in figs. 5 and 10. Such would furnish a ready and efficient channel of transportation between carpel and peduncle, so that the development or absence of the vertex of the inverted V would be of no physiological consequence, and its disappearance would not affect the plant's activities. Thus all of the unusual features of the vascular anatomy of a flower of *Calycanthus* can be explained upon the basis of a checked apical growth of the floral primordium.

PHYLOGENETIC POSITION.—In the description of the wild uncultivated form of *Caltha palustris* (17) it was shown that the frayed termini of the axial strands wander toward the apex of the conic receptacle; the floral appendages are indefinite in number and spirally inserted; the carpels are three-trace appendages; the stamens single-trace appendages; and the sepals three-trace appendages (but in the closely related *Clematis* either three or four traces). If one will picture the vascular skeleton of such a flower of the *Caltha* group of the Ranunculaceae with the apex of the receptacle indented, as one would push in the finger of a glove (due to the arrested growth of the apical primordium), one duplicates the vascular skeleton of the flower of the Calycanthaceae; therefore the Calycanthaceae are a specialized offshoot of the Ranunculaceae in which the growth of the apical primordium of the flower is delayed.

ORIGIN OF INFERIOR OVARY.—The study of *Calycanthus* arouses interest in a further consideration of the inferior ovary. Morphological and taxonomic evidence points to the inferior ovary as having arisen from several different centers of development. Has the inferior ovary always arisen after the same fashion? From the standpoint of vascular anatomy, such records as do exist (together with the author's own unpublished data upon the vascular anatomy of *Potentilla*, the cultivated roses, and *Sassafras*) indicate that the inferior ovary is due to a complete adnation and cohesion of the outer floral appendages to one another and to the ovary. *Calycanthus*, on the other hand, suggests the possibility that the inferior ovary could arise, due to a checking of the growth of the apical portion of the floral primordium, a bulging up of the surrounding meristematic tissue, and a subsequent fusion of this overhanging axial tissue with the carpel. Therefore the problem of the mode or modes of formation of the inferior ovary merit further and careful investigation from the standpoint of vascular anatomy.

CORTICAL VASCULAR SYSTEM.—The cortical vascular system of the flower differs from the cortical vascular system of the stem in several respects. For instance, the cortical bundles of the stem of *Calycanthus* (21) are inversely oriented, whereas those of the flower are not. Again the leaves and the leaf traces are arranged oppositely, whereas the floral appendages and floral traces are spirally

arranged. A third difference is that all of the traces to the lowermost perianth parts enter into the cortical system, but in the case of leaves the two lateral traces drop down through the cortex and the median leaf trace enters directly into the axial cylinder. The fourth difference is that all the traces from the higher perianth parts drop down through the cortical region slightly before passing into the vascular cylinder. Thus the traces from floral parts fail to form a continuous cortical system, as all of these traces ultimately pass into the axial cylinder; whereas in the vegetative part of the plant only the median leaf trace enters the axial cylinder, and the lateral leaf traces form a definite, continuous cortical system.

Whether the cortical vascular system of this floral axis represents a development independent of that of the vegetative axis (a less highly developed stage of such a development) or the remnants of such a condition cannot be determined; nevertheless a cortical vascular cylinder, in addition to the axial cylinder, denotes a derived condition. Therefore additional evidence is at hand to show that the flowers of the Calycanthaceae are a very specialized offshoot of a more primitive type, namely, the Ranunculaceae.

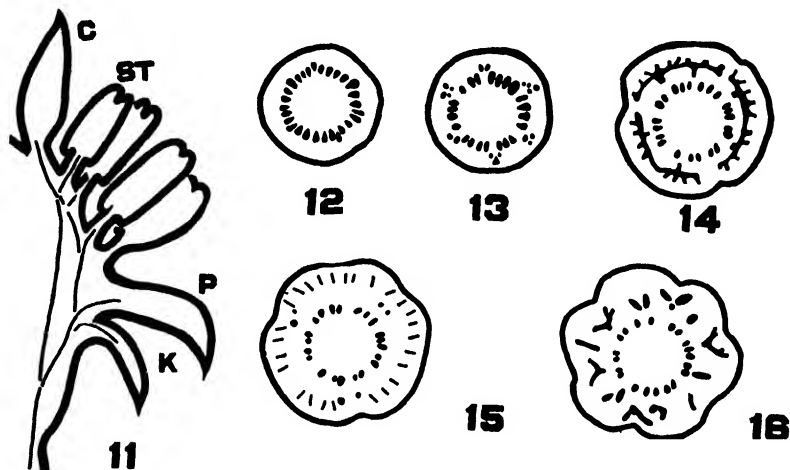
A summary of the preceding points is as follows. (1) *Calycanthus* has become specialized from forms having numerous perianth parts morphologically indistinguishable from one another, each receiving three or four traces, numerous single trace stamens, numerous three-trace carpels, and all appendages inserted in a strictly ascending spiral. (2) A suggestion of a cortical vascular system is present. (3) The hollow cuplike shape of the flower is due to a checking of the growth of the apical primordia, thereby giving the flower its inversely conic shape. (4) *Calycanthus* gives indication that the inferior ovary could be derived through a checking of the growth of the apical primordia of the receptacle, accompanied by a consequent bulging up of the surrounding meristematic tissue and a subsequent fusion of this overarching tissue with the ovary wall. On the other hand, the literature of floral vascular anatomy hints that the more common method is by adhesion and cohesion of floral appendages to one another.

#### **Annonaceae**

In the groups of the Ranales already investigated (Ranunculaceae, Menispermaceae, and Calycanthaceae) the vascular skeleton

is fundamentally of the same character. Does *Asimina triloba* Dunal. show the same characteristics of floral vascular anatomy as the three families just mentioned?

**ANATOMY OF FLOWER.**—In the pedicel the axial cylinder of vascular tissue consists of about thirty small wedge-shaped strands (fig. 12). In the lowest part of the receptacle some of the strands depart from six to eight points on this cylinder of small wedge-shaped strands toward the periphery. Usually six of these points are op-



FIGS. 11-16.—*Asimina triloba*: fig. 11, median longitudinal section of flower; figs. 12-16, cross-sections of flower at peduncle (12), sepal (13-15).

posite the median radii of the six petals. Either one small wedge-shaped strand departs from a point, or a group of two to four strands depart (fig. 13). No consistent plan is followed. As these strands pass upward and outward they sometimes gradually reverse their orientation. The change is most pronounced where groups of two to four strands leave the axial cylinder at one point. As these groups approach the sepal level they spread laterally in the cortical region of the receptacle, and fuse so that three arcs of tissue are formed (fig. 14). These arcs then break up into individual strands, all of which, except the end ones, pass outward as the traces to the sepals (fig. 15), thus forming the entire vascular supply to the first whorl of perianth parts. The strands forming the two extreme portions of

these arcs bend upward to form six to eight distinct cortical strands (fig. 15). Six of these are always on radii alternate with the petals, and at the petal level subdivide, so that the majority of the resulting strands pass outward and enter the six petals as the lateral traces to these appendages (fig. 16). The anatomical evidence indicates that all six petals are inserted at one level, rather than in two whorls of three each. The remaining cortical strands advance upward to higher levels; then some pass outward and enter the stamens, one to a stamen (fig. 11); others orient themselves so that they tie together the axial cylinder and the cortical vascular tissue (fig. 11). The petals, it has been stated, receive their lateral traces from the outer cortical cylinder, and they receive the median traces directly from the axial cylinder of small wedge-shaped strands. Thus the petals receive numerous traces; a portion of these are derived from the cortical cylinder and a portion from the axial cylinder. Some of the lower stamens receive their supply from the cortical cylinder, whereas others receive theirs from the axial cylinder of small wedge-shaped strands (fig. 11). A strand which passes outward from the cylinder toward a stamen may pass unbranched to a stamen, or may branch within the cortical region of the receptacle into from two to four strands and each strand pass to a stamen as its entire supply. Within the stamen a trace passes unbranched up the filament and connective. As it approaches the tip of the connective it flares into a fan-shaped series of strands and disappears. The orientation of some of the stamen traces which pass an appreciable distance upward through the receptacle becomes partially or entirely inverted. Above the stamen level the vascular cylinder of wedge-shaped strands breaks up into unit groups of three strands each; these units enter the individual carpel as one dorsal and two ventral traces. Within the carpel the dorsal trace sends forth a few lateral branches as it advances to the stigmatic region. Each ventral trace gives rise to an individual strand to each ovule of the row beneath it, and at the same time gives rise to a few dorsally directed branches.

#### DISCUSSION

The vascular system of the floral axis of *Asimina triloba* shows certain features akin to the cortical vascular system of a vegetative

axis. Many of the stamen traces drop down through the cortex and join other stamen traces at lower levels, just as leaf traces may drop down through the cortex. It is this condition which leads to the misconception that "a trace which passes outward from the axial vascular cylinder may branch within the cortical region of the receptacle, into from two to four strands and each strand pass to a stamen as the entire supply to that stamen." The condition is rather the reverse; several separate traces are fused to form a single cord. An even more striking likeness to the cortical vascular system of a vegetative axis is found in the petal region of the flower. The median petal traces pass directly to the axial cylinder, whereas the lateral petal traces drop down through the cortex and join lateral sepal traces, just as lateral leaf traces may drop down and unite with other lateral leaf traces. Here the similarity ceases. All of the sepal traces, both median and lateral, drop down through the cortex, but ultimately enter the axial vascular cylinder, thereby terminating the cortical system. It is also interesting to note that some of the cortical strands reverse their orientation as they drop down through the cortex. This again is a feature of cortical vascular systems. Thus a tendency, although not uniformly expressed by all of the individual floral appendages, exists toward the formation of a cortical vascular cylinder of tissue. A study of other members of the family should add more interesting data.

Since a tendency toward the formation of a cortical vascular system also exists in the flower of *Calycanthus floridus*, comparisons with that flower are interesting. In *Asimina* all perianth traces ultimately enter the axial vascular system, whereas in *Calycanthus* the trace to all of the lower perianth segments enters the cortical system, and all of the traces to the upper perianth parts enter the axial cylinder. Thus, in addition to the fact that the shape of the flower differs, the two cortical vascular systems have no definite common characteristics. This would indicate that the two cortical vascular systems have developed independently, and it may also indicate that the perianths of *Asimina* and *Calycanthus* are not homologous structures. The study of other members of the family should throw further light upon this subject.

The carpels reveal no conditions not already discussed in the

Ranunculaceae. They are of the primitive three-trace multiovulate type.

The stamens, as in all of the other families studied, are distinctly single-trace appendages. One peculiarity exists, however, which makes it desirable that other members of the family be studied. As has been said, some of the stamen traces pass directly inward to the axial cylinder, and others drop downward and join other stamen or petal traces, thereby forming a cortical vascular system. It was first thought that a regular alternation of axial and cortical traces existed. If it had been found that the stamens were so grouped that a stamen having a trace passing directly to the axial cylinder was flanked on each side by a stamen with its trace passing to the cortical system, then the situation would have been interesting. In the first place, every group of three stamens could have been taken to be a single unit, analogous to many vegetative axes in which the lateral traces of a leaf are cortical and the median trace is axial. In the second place, it still remains to be proved that every stamen with its anther and filament represents all of a single sporophyll. In the third place, evidence is accumulating that in one and perhaps a second of the remaining families of the Ranales three-trace and therefore presumably primitive stamens do exist. Thus the mere fact that an irregularity in the behavior of stamen traces of *Asimina triloba* does exist suggests that a further investigation of the family is desirable.

The petals of *Asimina* differ from the petals of the Ranunculaceae in two respects. They receive numerous traces, and the lateral traces are derived from a cortical vascular system. The first difference, numerous traces, definitely removes any staminal affinity, whereas the strictly single-trace condition of the petals of the Ranunculaceae bespeaks an undoubted staminal affinity of these petals. The second difference may be taken as significant of a foliar origin of the petals of *Asimina*, because the formation of a cortical system by the lateral traces is a characteristic sometimes displayed by leaves. So again a further study of the family will be interesting, especially a study of forms in which one whorl of petals is missing, reduced to mere scales, or is large and leathery.

The sepals differ from the sepals of the Ranunculaceae in that a

multitrace condition exists, and in that the traces have a tendency to pass cortically before entering the axial cylinder. Thus another question arises: Is this a characteristic of *Asimina triloba* alone or of the entire family? Only further investigations can answer this question.

An analysis of the data presented concerning the vascular anatomy of the flower of *Asimina triloba* reveals that it differs from the Ranunculaceae primarily in the development of a more or less definite cortical system of vascular strands, and in the foliar rather than staminal affinity of the petals. Because of these features a study of other members of the family is very desirable.

### Summary

The important features of vascular anatomy of the flower of certain unusual forms of *Ranunculus*, *Caltha*, and *Delphinium*, together with the important features of floral vascular anatomy of the families Menispermaceae, Calycanthaceae, and Annonaceae have been presented. The facts pertinent to each of these groups have been discussed and summarized at the end of each section; but a general concluding summary of the features common to all four groups is necessary. The floral organization of these groups supports the view already expressed, that in the more primitive flower the carpels are three-trace and multiovulate; the stamens are single-trace structures, and the lowest may be normally and naturally in the form of petals, or all may be transformed into petals by horticultural manipulation; the sepals are three-trace trilacunar structures, but may become modified into single trace unilacunar or multitrace, multilacunar structures; all floral parts are spirally inserted and indefinite as to number. As regards the origin of the Menispermaceae, Calycanthaceae, and Annonaceae, the vascular skeleton of each gives undoubted evidence of origin from the Ranunculaceae. The study of the vascular skeleton of the flower of *Menispermum canadense* shows that it has been derived directly from some member of the Ranunculaceae which displayed a tendency toward cyclic arrangement of parts, the reduction of parts to a definite numerical plan, a uniovulate carpellary condition, and a single strand vascular supply to the individual appendage. At the same time the Menispermaceae



demonstrate that they do not lead to any advanced group. The definite development of the imperfect flower makes this impossible. The floral vascular skeleton of the Calycanthaceae demonstrates that this family is also a diverging side line from the Ranunculaceae. The receptacle has become cup-shaped, due to a checking of the growth of the apical (carpellate portion) floral primordium and a consequent bulging up of the surrounding floral primordium to form the cup. Such a condition illustrates a possible step in the mode of origin of the inferior ovary. However, the Calycanthaceae, as the Menispermaceae, are a closed line because the anatomical evidence at hand points to the inferior ovary of the Rosaceae and other dicotyledons as arising through a cohesion and adhesion of floral parts, rather than after the fashion suggested by *Calycanthus*. The floral vascular skeleton of the Annonaceae is clearly that of the Ranunculaceae, but possessing one peculiarity, a partially developed cortical vascular system. This characteristic is seldom found in a vegetative axis, and has never been found in another floral axis except possibly the Calycanthaceae; therefore the Annonaceae also represent a closed series. Thus the evidence gained from a study of these three families points to the Ranunculaceae as the crucial center of the order.

The writer expresses his appreciation to Professors A. J. Eames of Cornell University and WM. TRELEASE of University of Illinois for their interest in this work. The double flowers of *Caltha* and *Ranunculus* were from the floricultural gardens of the University of Illinois, and the flowers of *Asimina triloba* were from Professor R. C. FRIESNER of Butler College.

BIOLOGICAL ABSTRACTS  
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## FURTHER AGGLUTINATION TESTS WITH BACTERIAL PLANT PATHOGENS

### I. BACTERIUM CAMPESTRE-BACT. PHASEOLI GROUP; BACT. MEDICAGINIS VAR. PHASEOLICOLA; BACT. TUMEFACIENS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 378

GEO. K. K. LINK AND ADELINE DES. LINK<sup>1</sup>

#### Introduction

In recent papers, SHARP (7) and LINK and SHARP (5) have reported success in differentiating bacterial plant pathogens by the agglutination test. This test was applied by them to three yellow organisms which are not readily differentiated culturally, but which exhibit marked host specificity; namely, *Bacterium campestre* (a crucifer pathogen), *Bact. phaseoli* (a bean pathogen), and *Bact. phaseoli sojense* (a soy bean pathogen). It was also applied to a yellow organism, *Bact. flaccumfaciens*, which attacks the same host as *Bact. phaseoli*, but which causes quite distinct symptoms and also is culturally distinguishable from it. It was concluded that serological specificity is correlated with host specificity for *Bact. campestre*, *Bact. phaseoli*, and *Bact. phaseoli sojense*, and with symptom specificity for *Bact. phaseoli* and *Bact. flaccumfaciens*, and that the designation of these organisms as distinct species is justifiable serologically. It was also concluded that these organisms, while distinct, nevertheless are closely related.

The first part of the experiments reported here is a continuation and enlargement of the study of the yellow organisms of the so-called *Bact. campestre*-*Bact. phaseoli* group of SMITH (8). It was our plan to complete the agglutination studies of this group, which, in addition to *Bact. campestre* (black rot of crucifers), *Bact. phaseoli* (blight of beans), and *Bact. phaseoli sojense* (pustule of soy bean), might include *Bact. malvacearum* (angular leafspot of cotton),

<sup>1</sup> The Department of Hygiene and Bacteriology cooperated in providing space for the animals, and Dr. W. H. TALIAFERRO and Dr. L. G. TALIAFERRO of that department assisted us throughout the course of the experiments.

*Bact. citri* (citrus canker), *Bact. pruni* (blackspot and canker of plum and peach), *Bact. juglandis* (walnut blight), *Bact. translucens* (blight of barley), and possibly others. Of the latter we were able to obtain cultures of only *Bact. malvacearum*, *Bact. citri*, and *Bact. pruni*. We received, however, from Dr. E. F. SMITH cultures of *Bact. cucurbitae* (leafspot of squash), and also of an unnamed yellow organism which he asked us to test and which is here designated as *Bact. X*. Cultures of *Bact. citri*, *Bact. cucurbitae*, *Bact. pruni*, and *Bact. X* were not received until after immunization of the animals had begun, and as a result we were not able to make tests with their antisera.

The second part of the experiments here reported grew out of SHARP'S study (7) of bean pathogens. In addition to those tested by him, namely, *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens*, which are serologically closely related but distinct, we used *Bact. medicaginis* var. *phaseolicola*, a white organism which causes symptoms in the bean practically identical with those caused by *Bact. flaccumfaciens*, but which is culturally distinct from the latter and is closely related to *Bact. medicaginis* (2). This group of tests was made to determine whether *Bact. medicaginis* var. *phaseolicola* can be differentiated serologically from the other bean pathogens, and also to ascertain what serological relation, if any, it bears as a representative of the *Bact. medicaginis* group to the *Bact. campestre*-*Bact. phaseoli* group.

After the conclusion of the experimental work here reported, a paper of BROOKS, NAIN, and RHODES (1) which has bearing on this problem came to our attention. They applied the agglutination test to the following yellow organisms: *Bact. campestre*, *Bact. malvacearum*, *Bact. michiganense*, *Bact. pruni*, *Bact. phaseoli*, *Bact. phaseoli sojense*, *Bact. gummisudans*, *Bact. stewartii*, *Bact. pelargoni*, *Bact. vitians*, and *Bact. tracheiphilus*. The agglutinability of these organisms was tested in 1:100 dilutions of sera with titres of 1:8000. They concluded that a close relationship exists between *Bact. campestre* (certain strains), *Bact. malvacearum*, *Bact. phaseoli*, *Bact. phaseoli sojense*, *Bact. pelargoni*, and *Bact. vitians*. On the other hand, *Bact. michiganense*, *Bact. pruni*, *Bact. stewartii*, *Bact. tracheiphilus*, *Bact. hyacinthi*, and *Bact. lathyri* have no relationship to one another or to other members of the yellow group. One strain of

*Bact. campestre* stood by itself with regard to all the other organisms studied.

In order to put to an even more rigorous trial the suitability of the direct agglutination test as a means of differentiating and identifying bacterial plant pathogens, we included *Bact. tumefaciens* (crown gall pathogen), and tested it against the yellow organisms as well as against *Bact. medicaginis* var. *phaseolicola*. JENSEN (3), using the agglutination test, demonstrated that the American and Danish daisy strains of *Bact. tumefaciens* are distinct serologically. RIKER (6) reported success in developing in rabbits an antiserum of *Bact. tumefaciens*, with a titre of 1-3000.

In part II are reported agglutination experiments with members of the soft-rot group, *Bacillus aroideae* and *B. carotovorus*.

#### Sources of material

The cultures of *Bact. campestre*, *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens* were of the same stock as those used by SHARP (7) and LINK and SHARP (5). From Dr. J. G. BROWN we obtained a culture of *Bact. malvacearum*, the pathogenicity of which was tested before the experiment. Cultures of *Bact. citri*, *Bact. cucurbitae*, *Bact. pruni*, and *Bact. X* were supplied by Dr. E. F. SMITH's laboratory; their pathogenicity was not tested by us. Dr. W. H. BURKHOLDER supplied us with a culture of *Bact. medicaginis* var. *phaseolicola* whose pathogenicity we did not test. The culture of *Bact. tumefaciens* came from isolations made from tomato galls produced by inoculation with a culture supplied us by Dr. A. J. RIKER in 1925.

#### Experimental procedure

Each organism was grown on standard potato-dextrose agar slants (pH 7.2) for four days. Even suspensions were then made of each organism in 50 cc. of 0.85 per cent NaCl solution. These suspensions were used as stock antigens for injections of rabbits and production of antisera. Two animals were used for each organism, so that the tests were run in duplicate. Each of the animals used was bled from the heart before the first injection, so that the serum might be tested for the presence of natural antibodies against all of the organisms to be tested later.

At first, following the method used by LINK and SHARP, we

injected suspensions of live organisms intravenously into the ear-vein of the animals. This method was successful with most of the animals but was fatal to some, especially to those injected with organisms of the soft-rot group. We tried intraperitoneal injections with these organisms, and found that they were less likely to be fatal to the animals. As a result, most of the antisera used in these experiments were obtained by injecting suspensions of live organisms intravenously five times into the ear-vein of the animals, at intervals of three days, using 0.5 cc. of the suspension for the first and 1 cc. for each of the remaining four injections; while a few of the antisera were obtained by intraperitoneal injections, giving 1 cc. of suspension of live organisms in the first injection and 2 cc. in each of two subsequent injections, with three-day intervals. The antisera produced by intraperitoneal injections are starred in the protocols. Seven days after the last injection the animals were bled from the heart, and the sera prepared and kept aseptically until they were used in the agglutination tests.

The cultures used for the preparation of suspensions in the agglutination tests were grown on standard beef extract-peptone-dextrose agar (pH 7.2). The organisms were washed off the agar with 0.85 per cent salt solution, and then shaken thoroughly in saline solution and centrifugated. The supernatant liquid was decanted, and the organisms were washed and centrifugated a second time. The last suspension in saline solution was used for the agglutination tests. The organisms were grown on different media, and washed in saline to take precaution against a positive precipitin reaction with the protein of the potato agar used in preparing the stock antigens.

Observations made in the course of tests with suspensions of the organisms against the normal sera led us to modify our procedure of making suspensions for the tests with the antisera. When we read the agglutination tests of the normal sera against suspensions in saline of *Bact. tumefaciens*, we found that there had been flocculation in all tests at all dilutions, but that most of the suspended organisms were still in suspension. The controls in saline behaved identically. Examination of the excess stock suspension which had been kept in the ice chest over night revealed that in this, too, there had been flocculation of a fraction of the organisms. Suspensions of *Bacillus*

TABLE I  
TYPICAL PROTOCOL OF AGGLUTINATION TESTS

	Tubes										Con- trol
	1	2	3	4	5	6	7	8	9	10	
Antiserum } Dilution.....	1-5	1-10	1-20	1-40	1-80	1-160	1-320	1-640	1-1280	1-2560	.....
Antiserum } Amount in cc.....	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0
Bacterial suspension (antigen) in cc.....	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Saline solution (0.85 per cent NaCl).....	0	0	0	0	0	0	0	0	0	0	0.5
Final dilution of antiserum.....	1-10	1-20	1-40	1-80	1-160	1-320	1-640	1-1280	1-2560	1-5120	.....

*carotovorus* 3a and *B. aroideae* behaved similarly, but the settling was relatively slight, although it occurred in all tubes and in the saline control. Consequently, for the later tests of antisera against suspensions of *Bact. tumefaciens*, *B. carotovorus* 3a, and *B. aroideae* we separated the readily flocculating fraction by centrifugation at low speed, allowed the suspension to stand over night in the ice chest, and then pipetted off the supernatant suspension and used this in the agglutination tests. This method gave us suspensions which did not come down in the saline control. The possible significance of these findings has previously been discussed (4).

The sample protocol of table I shows the set-up used in each experiment. 0.2 cc. of antiserum was added to 0.8 cc. of saline solution (0.85 per cent NaCl), giving a dilution of 1-5. From this original dilution of 1-5 the other dilutions were obtained by mixing and then transferring 0.5 cc. of the diluted antiserum from tube 1 to tube 2, which contained 0.5 cc. of saline, from tube 2 to tube 3, and so on, up through tube 10 or 12. After thoroughly mixing the contents of each tube, 0.5 cc. of bacterial suspension in saline solution (antigen) was added to each tube, thus giving a dilution of 1-10 in tube 1, up through dilution 1-5120 in tube 10. The control in each set-up consisted of a tube containing 0.5 cc. saline and 0.5 cc. of bacterial suspension in saline. After thorough mixing by shaking, the series of tubes was incubated for one hour at 37° C. in a Wassermann bath, placed in a refrigerator for twelve hours, and then read both macroscopically and with a hand lens.

As a control for all series, a sample of serum drawn before immunization of each animal was tested against suspensions of the organisms to be used in tests against the antisera obtained from the animal. The set-up for these experiments was identical with that just described, save that normal serum (drawn before immunization) was used in place of antiserum.

The results of these experiments are presented in tables II to IX. The protocols of the tests for the presence of natural antibodies in the normal sera of the animals used are not given in detail. They are in each case identical with the protocols of the tests with the antisera; in other words, each protocol stands for two tests, one with the normal serum and the other with the antiserum of the animal against suspensions of the same organisms.



TABLE II\*  
ANTISERUM OF BACTERIUM CAMPESTRE AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CON- TROL
		1	2	3	4	5	6	7	8	9	10	
Bact. campestre.....	{ 87 154	+++ .....	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	—
Bact. citri.....	{ 87 154	— .....	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
Bact. cucurbitae.....	{ 87 154	— .....	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
Bact. malvacearum.....	{ 87 154	++ —	++ —	— —	— —	— —	— —	— —	— —	— —	— —	—
Bact. pruni.....	{ 87 154	— .....	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
Bact. X.....	{ 87 154	+++ .....	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	—
Bact. medicaginis var. phasecolica.....	{ 87 154	— .....	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	—
Bact. tumefaciens.....	{ 87 154	+++ .....	+++ —	+++ —	+++ —	+++ —	+++ —	+++ —	+++ —	+++ —	+++ —	—

\* In table II and all the following tables where the symbols are used, +++ stands for complete clearing of the suspension with total deposition in a clump; ++ stands for complete clearing but only partial deposition in a clump, many clumps being in suspension; + stands for partial clearing with a small clump and some clumps in suspension; — stands for partial clearing with a very small clump and a few clumps in suspension; and — stands for no change in turbidity. All readings were made macroscopically as well as with a hand lens.

In control tests with the sera of these animals before immunization, all tests were negative excepting those against suspensions of *Bact. tumefaciens*. These gave partial agglutination in all dilutions of sera as well as in the saline control (see page 181).

TABLE III\*  
ANTISERUM OF BACT. MALVACEARUM AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CON- TROL
		1	2	3	4	5	6	7	8	9	10	
Bact. campestre.....	{ 128† 168	-	-	-	-	-	-	-	-	-	-	-
Bact. citri.....	{ 128 168	-	-	-	-	-	-	-	-	-	-	-
Bact. cucurbitae.....	{ 128 168	-	-	-	-	-	-	-	-	-	-	-
Bact. malvacearum.....	{ 128 168	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	-	-	-
Bact. phaseoli.....	{ 128 168	- ++	++ ++	++ ++	++ ++	++ ++	++ ++	- ++	- ++	-	-	-
Bact. phaseoli sojense (S)	{ 128 168	-	-	-	-	-	-	-	-	-	-	-
Bact. pruni.....	{ 128 168	-	-	-	-	-	-	-	-	-	-	-
Bact. X.....	{ 128 168	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++
Bact. flaccumfaciens.....	{ 128 168	-	-	-	-	-	-	-	-	-	-	-
Bact. medicaginis var. phaseolicola.....	{ 128 168	-	-	-	-	-	-	-	-	-	-	-
Bact. tumefaciens.....	{ 128 168	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++

\* For data of the control tests with sera of these animals before immunization, see footnote of table II.

† Injected intraperitoneally.

TABLE IV\*  
ANTISERUM OF BACT. PHASEOLI AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CON- TROL
		1	2	3	4	5	6	7	8	9	10	
Bact. citri.....	{ 169 170	-	-	-	-	-	-	-	-	-	-	-
Bact. cucurbitae.....	{ 169 170	-	-	-	-	-	-	-	-	-	-	-
Bact. malvacearum.....	{ 169 170	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	++ ++	-	-	-	-
Bact. phaseoli.....	{ 169 170	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	-
Bact. pruni.....	{ 169 170	-	-	-	-	-	-	-	-	-	-	-
Bact. X.....	{ 169 170	+++ +++	+++ +++	+++ +++	+++ +++	++ ++	-	-	-	-	-	-
Bact. medicaginis var. phaseolicola.....	{ 169 170	+++ +++	+++ +++	+++ +++	+++ +++	-	-	-	-	-	-	-
Bact. tumefaciens.....	{ 169 170	+++ +++	+++ +++	+++ +++	+++ +++	++ ++	-	-	-	-	-	-

\* For data of the control tests with sera of the animals before immunization, see footnote of table II.

TABLE V\*  
ANTISERUM OF BACTERIUM PHASEOLI SOJENSE (S) AGAINST

SUSPENSION OF (ANTIGEN)	ANTIAL	TUBES										CON- TROL
		1	2	3	4	5	6	7	8	9	10	
Bact. citri.....	{ 100† 161	-	-	-	-	-	-	-	-	-	-	-
Bact. cucurbitae.....	{ 100 161	-	-	-	-	-	-	-	-	-	-	-
Bact. malvacearum.....	{ 100 161	-	++	++	++	++	++	++	++	++	++	-
Bact. phaseoli sojense (S)	{ 100 161	++	++	++	++	++	++	++	++	++	++	-
Bact. pruni.....	{ 100 161	-	-	-	-	-	-	-	-	-	-	-
Bact. X.....	{ 100 161	++	++	++	++	++	++	++	++	++	++	-
Bact. medicaginis var. phaseolicola.....	{ 100 161	-	++	++	++	++	++	++	++	++	++	-
Bact. tumefaciens.....	{ 100 161	++	++	++	++	++	++	++	++	++	++	-

\* For data of the control tests with sera of the animals before immunization, see footnote of table II.

† Injected intraperitoneally.

TABLE VI\*  
ANTISERUM OF BACT. PHASEOLI SOJENSE (R) AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CON- TROL
		1	2	3	4	5	6	7	8	9	10	
Bact. citri.....	{ 139† 172	-	-	-	-	-	-	-	-	-	-	-
Bact. cucurbitae.....	{ 139 172	-	-	-	-	-	-	-	-	-	-	-
Bact. malvacearum.....	{ 139 172	++	++	++	++	++	++	++	++	-	-	-
Bact. phaseoli sojense (R)	{ 139 172	++	++	++	++	-	-	-	-	-	-	-
Bact. pruni.....	{ 139 172	-	-	-	-	-	-	-	-	-	-	-
Bact. X.....	{ 139 172	++	++	++	++	-	-	-	-	-	-	-
Bact. medicaginis var. phaseolicola.....	{ 139 172	++	++	++	-	-	-	-	-	-	-	-
Bact. tumefaciens.....	{ 139 172	++	++	++	+	+	+	-	-	-	-	-

\* For data of the control tests with sera of the animals before immunization, see footnote of table II.

† Injected intraperitoneally.

TABLE VII\*  
ANTISERUM OF BACTERIUM FLACCUMFACIENS AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CON- TROL
		1	2	3	4	5	6	7	8	9	10	
Bact. citri.....	{ 156 157	-	-	-	-	-	-	-	-	-	-	-
Bact. cucurbitae.....	{ 156 157	-	-	-	-	-	-	-	-	-	-	-
Bact. malvacearum.....	{ 156 157	++ ++	++ -	-	-	-	-	-	-	-	-	-
Bact. pruni.....	{ 156 157	-	-	-	-	-	-	-	-	-	-	-
Bact. X.....	{ 156 157	++ -	++ -	+	-	-	-	-	-	-	-	-
Bact. flaccumfaciens.....	{ 156 157	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	-	-	-	-
Bact. medicaginis var. phaseolicola.....	{ 156 157	++ +	++ ++	++ ++	++ ++	++ +	-	-	-	-	-	-
Bact. tumefaciens.....	{ 156 157	++ ++	++ ++	+	-	-	-	-	-	-	-	-

\* For data of the control tests with sera of the animals before immunization, see footnote of table II.

TABLE VIII\*  
ANTISERUM OF BACT. MEDICAGINIS VAR. PHASEOLICOLA AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CON- TROL
		1	2	3	4	5	6	7	8	9	10	
Bact. campestre.....	164†	—	—	—	—	—	—	—	—	—	—	—
Bact. citri.....	164	—	—	—	—	—	—	—	—	—	—	—
Bact. cucurbitae.....	164	—	—	—	—	—	—	—	—	—	—	—
Bact. malvacearum.....	164	—	—	—	—	—	—	—	—	—	—	—
Bact. phaseoli.....	164	—	—	—	—	—	—	—	—	—	—	—
Bact. phaseoli sojense (S)	164	—	—	—	—	—	—	—	—	—	—	—
Bact. pruni.....	164	—	—	—	—	—	—	—	—	—	—	—
Bact. X.....	164	—	—	—	—	—	—	—	—	—	—	—
Bact. flaccumfaciens.....	164	—	—	—	—	—	—	—	—	—	—	—
Bact. medicaginis var. phaseolica.....	164	++	++	++	++	++	++	++	—	—	—	—
Bact. tumefaciens.....	164	++	++	++	++	++	++	++	—	—	—	—

\* For data of the control tests with sera of the animals before immunization, see footnote of table II.

† The second animal died after the last injection.

TABLE IX\*  
ANTISERUM OF BACT. TUMEFACIENS AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CON- TROL
		1	2	3	4	5	6	7	8	9	10	
Bact. campestre . . . . .	{ 152 153	-	-	-	-	-	-	-	-	-	-	-
Bact. citri . . . . .	{ 152 153	-	-	-	-	-	-	-	-	-	-	-
Bact. cucurbitae . . . . .	{ 152 153	-	-	-	-	-	-	-	-	-	-	-
Bact. malvacearum . . . . .	{ 152 153	-	-	-	-	-	-	-	-	-	-	-
Bact. phaseoli . . . . .	{ 152 153	-	-	-	-	-	-	-	-	-	-	-
Bact. phaseoli sojense (S)	{ 152 153	-	-	-	-	-	-	-	-	-	-	-
Bact. pruni . . . . .	{ 152 153	-	-	-	-	-	-	-	-	-	-	-
Bact. X . . . . .	{ 152 153	-	-	-	-	-	-	-	-	-	-	-
Bact. flaccumfaciens . . . . .	{ 152 153	-	-	-	-	-	-	-	-	-	-	-
Bact. medicaginis var. phaseolicola . . . . .	{ 152 153	-	-	-	-	-	-	-	-	-	-	-
Bact. tumefaciens . . . . .	{ 152 153	++	++	++	++	++	++	++	++	++	++	++

\* For data of the control tests with sera of the animals before immunization, see footnote of table II.



### Discussion

In the tests of the normal sera, no agglutination was noted aside from that which occurred in all tubes which contained suspensions of *Bact. tumefaciens*, *B. carotovorus* 3a, or *B. aroideae*. That this agglutination was spontaneous and not due to the action of the normal sera is proved by the observations: (1) that it occurred equally in the control tubes containing only suspensions and saline; (2) that it did not occur in the saline controls in the second test in which suspensions were used from which the spontaneously agglutinating portion had been removed; and (3) that in the second test with the antisera of the same animals against the same organisms, no agglutination occurred in the higher dilutions in some instances.

In most cases three intraperitoneal injections (the first 1 cc. and the others 2 cc.) produced antisera with practically the same titres as five intravenous injections into the ear-vein (the first 0.5 cc. and the others 1 cc.). It is fortunate that antisera against plant pathogens can be produced by intraperitoneal injections, because this method is easier of manipulation and less likely to be fatal to animals than that of intravenous injection. Of the twenty-three animals injected intravenously eleven died, while of the eight injected intraperitoneally only two died. The only exception occurred with *Bact. malvacearum*. This organism when injected intravenously produced an antiserum which gave a titre of 1-1280 against suspensions of the homologous organism, but when injected intraperitoneally produced an antiserum which gave a titre of only 1-10 against its own suspensions (table III).

In the main the titres of the antisera of the two animals injected with the same organism are of the same magnitude. That there are idiosyncracies resident in individual animals is brought out in several cases. Thus (table II) the antiserum of *Bact. campestre* of one animal gave a titre against suspensions of *Bact. medicaginis* var. *phaseolicola*, *Bact. malvacearum*, and *Bact. tumefaciens*, while that of the other animal did not. In two other instances the antiserum of one animal reacted against suspensions of *Bact. medicaginis* var. *phaseolicola*, while that of the other did not (tables V and VI). Similarly, the antiserum of *Bact. flaccumfaciens* of one animal gave a titre against

suspensions of *Bact. X*, whereas that of the other animal failed to do so (table VII).

As in the experiments previously reported by LINK and SHARP, *Bact. campestre* again produced an antiserum which was highly specific for its homologous organism (table II). This antiserum reacted almost identically with suspensions of *Bact. X*. Although the titre obtained against this organism was not as high and the agglutination was slightly less in nearly all dilutions, this test indicates that these organisms contain antigens which lead to production of the same antibodies, and that they probably are closely related. Unfortunately we did not have any antiserum of *Bact. X* to test against suspensions of itself and of *Bact. campestre*, and consequently no final statement can be made as to their relationship. The antiserum of *Bact. campestre* gave either no titre, or a lower titre with suspensions of other yellow organisms which are taken to belong to the *Bact. campestre*-*Bact. phaseoli* group (*Bact. citri*, *Bact. malvacearum*, *Bact. pruni*, and possibly *Bact. cucurbitae*) than it did against suspensions of *Bact. medicaginis* var. *phaseolicola* and *Bact. tumefaciens*. The reaction against *Bact. malvacearum* was stronger than that against *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens* (5, 7).

The antiserum of *Bact. malvacearum* gave specific agglutination against suspensions of itself, good agglutination (titre 1-320) against *Bact. phaseoli*, and a slight agglutination against suspensions of *Bact. X*. It did not give reactions with suspensions of any of the other yellow organisms, nor with *Bact. medicaginis* var. *phaseolicola*, but did give a low titre against suspensions of *Bact. tumefaciens*.

*Bact. phaseoli*, which had produced an antiserum giving specific agglutination against suspensions of the homologous organism, and also agglutination of a lower titre against suspensions of *Bact. campestre* (5), *Bact. phaseoli sojense* (S), and of *Bact. flaccumfaciens* (7), again produced an antiserum which was specific against suspensions of itself (titre 1-5120), complete agglutination occurring in one case through dilution 1-5120, and in the other case through dilution 1-2560. A very good titre was also obtained against suspensions of *Bact. malvacearum* and a lower one with *Bact. X*. This seems to indicate that, serologically at least, *Bact. phaseoli*, *Bact. malva-*

*cearum*, and *Bact. X* are related. No titres were obtained against suspensions of the other yellow organisms. Titres of 1-80 and 1-160 were obtained respectively against suspensions of *Bact. medicaginis* var. *phaseolicola* and *Bact. tumefaciens*.

SHARP (7) and LINK and SHARP (5) found the antiserum of *Bact. phaseoli sojense* (S) to be specific when tested against suspensions of the homologous organism and of *Bact. campestre*, *Bact. phaseoli*, and *Bact. flaccumfaciens*. In our tests the antisera of *Bact. phaseoli sojense* (S) and (R) gave lower titres against suspensions of the homologous organisms than against suspensions of *Bact. malvacearum*. This may be interpreted as indicating that, in addition to the antigens which produce antibodies against *Bact. phaseoli sojense* (S) and (R), these organisms contain also antigens which stimulate the production of the same antibodies as *Bact. malvacearum*. Absorption of agglutinins might have enabled us to use the antisera of *Bact. phaseoli sojense* (S) and (R) to differentiate specifically between these organisms and *Bact. malvacearum*. The antisera of *Bact. phaseoli sojense* (S) and (R) gave agglutination in low dilutions against suspensions of *Bact. X*. Agglutination in low dilutions occurred also when these antisera were tested against suspensions of *Bact. medicaginis* var. *phaseolicola* and *Bact. tumefaciens*.

The antiserum of *Bact. flaccumfaciens*, according to SHARP (7) and LINK and SHARP (5), gives specific agglutination against suspensions of the homologous organism, and slight agglutination against suspensions of *Bact. campestre*, *Bact. phaseoli*, and *Bact. phaseoli sojense*. In these experiments its antiserum was highly specific for its homologous organism, giving a titre of 1-640, with complete agglutination through dilution 1-320. It gave slight agglutination against suspensions of *Bact. malvacearum* and of *Bact. X* (antiserum of one animal only). Very slight agglutination was obtained against suspensions of *Bact. medicaginis* var. *phaseolicola*, which causes practically identical symptoms in the bean. Stronger agglutination was obtained against suspensions of *Bact. tumefaciens* (titre 1-40, complete agglutination in dilution 1-20).

The antiserum of *Bact. medicaginis* var. *phaseolicola* was highly specific for its homologous organism, giving complete agglutination through the dilution range 1-10 to 1-640, but giving partial aggluti-

nation with suspensions of *Bact. tumefaciens* in low dilutions (1-10 to 1-20).

*Bact. tumefaciens* produced an antiserum which was extremely specific for its homologous organism, giving complete agglutination through the dilution range 1-10 to 1-5120 in one case, and 1-10 to 1-1280 in the other, and no agglutination at all with suspensions of all the other organisms tested.

On the basis of current interpretations of many immunologists, these results may indicate that while *Bact. campestre*, *Bact. malvacearum*, *Bact. phaseoli*, *Bact. phaseoli sojense* (S), *Bact. phaseoli sojense* (R), and *Bact. flaccumfaciens* contain some antigens (proteins) which stimulate the production of antibodies that react with *Bact. tumefaciens*, this organism does not contain any antigens (proteins) which stimulate production of antibodies that react with the other organisms. On this basis, therefore, *Bact. tumefaciens* can be differentiated specifically from the other organisms studied. Similarly, *Bact. medicaginis* var. *phaseolicola* can be differentiated from all the other organisms, including the bean pathogens *Bact. phaseoli* and *Bact. flaccumfaciens*, even though *Bact. campestre*, *Bact. phaseoli*, *Bact. phaseoli sojense* (S) and (R), and *Bact. flaccumfaciens* contain antigens which stimulate to a slight extent the production of antibodies that react with *Bact. medicaginis* var. *phaseolicola*. In other words, this organism, which in beans causes symptoms identical with those generally caused by *Bact. flaccumfaciens* and occasionally by *Bact. phaseoli*, is not only morphologically and physiologically distinct from these organisms, as determined by culture, but is also serologically distinct. In fact, serologically, *Bact. medicaginis* var. *phaseolicola* seems to stand closer to *Bact. campestre* and *Bact. phaseoli* than it does to *Bact. flaccumfaciens*.

*Bact. malvacearum* contains antigens that stimulate production of the same antibodies as *Bact. phaseoli*, and, to a less extent, as *Bact. X*; however, it contains in addition antigens that stimulate the production of antibodies that react only with its own suspensions. *Bact. malvacearum* can therefore be separated from the other yellow organisms on this basis. Serologically it seems to be more closely related to *Bact. phaseoli*, *Bact. phaseoli sojense* (S) and (R), and *Bact. X* than to *Bact. campestre* and *Bact. flaccumfaciens*.

The reaction of *Bact. X* to the antibodies of the *Bact. campestre* antigens is almost identical with that of *Bact. campestre*.

*Bact. citri*, *Bact. cucurbitae*, and *Bact. pruni* did not react with the antibodies produced by the antigens of any of the other organisms of the so-called yellow *Bact. campestre*-*Bact. phaseoli* group.

This raises the question whether the yellow organisms grouped by SMITH really constitute a single group. The results of SHARP (7) and LINK and SHARP (5) indicate that *Bact. campestre*, *Bact. flaccum-faciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense*, although distinct, are closely related serologically. The experiments here reported indicate that *Bact. malvacearum*, while distinct, also belongs to this group. This is in harmony with the findings of BROOKS, NAIN, and RHODES (1), who, however, found a closer relationship between *Bact. malvacearum* and *Bact. campestre* than we did. Although our data are not conclusive, because no tests were made with antisera of *Bact. citri*, *Bact. cucurbitae*, and *Bact. pruni*, they indicate strongly that these yellow organisms do not belong in the *Bact. campestre*-*Bact. phaseoli* group. BROOKS, NAIN, and RHODES had found by reciprocal tests that *Bact. pruni* does not belong to the *Bact. campestre*-*Bact. phaseoli* group.

### Conclusions

1. The agglutination test can be used to differentiate *Bact. malvacearum* from the yellow organisms *Bact. campestre*, *Bact. phaseoli*, *Bact. citri*, *Bact. cucurbitae*, and *Bact. pruni*.

2. When the antiserum of *Bact. malvacearum* is tested against suspensions of *Bact. phaseoli sojense*, a specific differentiating test is obtained; when the antiserum of *Bact. phaseoli sojense* is used against suspensions of the homologous organism and of *Bact. malvacearum* the direct agglutination test is not specific and differentiating.

3. Serologically *Bact. malvacearum* is more closely related to *Bact. phaseoli* and *Bact. phaseoli sojense* than to *Bact. campestre*.

4. The yellow organisms tested probably are not a single group serologically. *Bact. campestre*, *Bact. malvacearum*, *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccum-faciens* apparently constitute a serological group.

5. *Bact. medicaginis* var. *phaseolicola* can be differentiated by the agglutination test from the bean pathogens *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens*.

6. When the antiserum of *Bact. tumefaciens* is tested against suspensions of the homologous and of various heterologous organisms, highly specific agglutination occurs; when the antisera of these heterologous organisms are tested against suspensions of *Bact. tumefaciens*, agglutination generally takes place in the lower dilutions.

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# FURTHER AGGLUTINATION TESTS WITH BACTERIAL PLANT PATHOGENS

## II. SOFT-ROT GROUP: *BACILLUS AROIDEAE* AND *B. CAROTOVORUS*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 379<sup>1</sup>

GEO. K. K. LINK AND W. H. TALIAFERRO

### Introduction

Several species of soft-rot bacteria have been described, but there has been no unanimity among investigators as to whether these are in fact distinct species. HARDING and MORSE (2) made extensive comparative studies of the soft-rot organisms, including *Bacillus aroideae*, *B. carotovorus*, *B. oleraceae*, and *B. omnivorus*, and thirty-nine so-called strains isolated from various hosts affected with soft-rot. They applied thirty-eight classificatory criteria, and concluded that the forty-three strains studied were alike except in their ability and manner of fermenting sugars. They placed *B. carotovorus*, *B. oleraceae*, *B. omnivorus*, and thirty of the unnamed strains into one group, on the basis of their forming acid and gas from dextrose, sucrose, and lactose; *B. aroideae* and three of the unnamed strains were put into a second group, on the basis of their forming acid but no gas from these sugars; the other six unnamed strains were considered intermediate between groups one and two. HARDING and MORSE felt that these differences were not sufficient to differentiate the organisms, and concluded that it was not advisable to consider *B. carotovorus* and *B. aroideae* distinct species. SMITH (5) considered *B. carotovorus*, *B. oleraceae*, and *B. omnivorus* apparently identical, and *B. aroideae* closely related to *B. carotovorus*. MASSEY (4), proceeding from the work of HARDING and MORSE and accepting their grouping of all soft-rot organisms as two species, *B. carotovorus* and *B. aroideae* (or preferably as one species, *B. carotovorus*), made a

<sup>1</sup> Joint contribution from this laboratory and from the Department of Hygiene and Bacteriology.

study of *B. carotovorus* and *B. aroideae*, using as criteria the hosts affected, and the action of these organisms on various organic carbon compounds. He found that calla, iris, kohlrabi, and cauliflower can be used as hosts to differentiate *B. carotovorus* from *B. aroideae*. *B. carotovorus* gave positive results only when inoculated into iris, while *B. aroideae* did not rot iris but produced rot when inoculated into calla, kohlrabi, and cauliflower. He verified the findings of HARDING and MORSE as to the action of these organisms upon sugars, and found further that they can be separated on the basis of their action upon ethyl alcohol media. He concluded that, although the organisms are closely related, they should be maintained as separate species. TAKIMATO (6) reported that serological tests indicate that the bacteria isolated from soft-rot of celery, lettuce, and radish belong to the same strain, although the radish organism differs from the others when grown in culture media.

We considered that it would be well to use the agglutination test to determine whether the host specificity reported by MASSEY for *B. aroideae* and *B. carotovorus* is correlated with serological specificity; that is, whether *B. aroideae* and *B. carotovorus* are serologically distinct. The experiments here reported consist of tests with *B. aroideae*, and with a strain of *B. carotovorus* here designated as *B. carotovorus* 3a. They also involve tests of these organisms against *Bact. campestris* of the *Bact. campestris*-*Bact. phaseoli* group, *Bact. medicaginis* var. *phaseolicola* of the *Bact. medicaginis* group, and *Bact. tumefaciens*.

After the conclusion of the experiments reported here, two papers with bearing upon this problem came to our attention. LACEY (3) made a study of the soft-rot organisms *B. carotovorus*, *B. solaniasaprus*, and *B. phytophthorus*, applying cultural, pathological, and serological tests. These showed that there is a close relationship between these organisms, but that they are sufficiently different "to warrant their continued separation into distinct species." Even though there was common group agglutination, the species could be separated by the agglutination test. BERRIDGE (1) applied chemical agglutination tests to *B. solaniasaprus*, *B. phytophthorus*, and *B. carotovorus*. She concluded that for the organisms of this group chemical agglutination tests are as reliable as serum agglutination tests. The chem-



ical agglutination tests show that *B. solanisaprus* and *B. phytophthorus* are not identical organisms, and are distinct from but closely related to *B. carotovorus*.

### Source of cultures

A culture of *Bacillus carotovorus* 3a was obtained from Dr. J. G. LEECH, and a culture of *B. aroideae* was secured from Dr. A. B. MASSEY. We did not test the pathogenicity of these organisms.

### Experimental procedure

The procedure was exactly that used in the experiments described in Part I; in fact, the first tests made on the soft-rot organisms were run at the same time as those reported in Part I, which should be consulted for the description of the experimental method and for the sample protocol. When the results of the first experiment with the soft-rot organisms were read, it was found that the dilutions had not been high enough to determine the titres in all cases; consequently some of each of the antisera was placed in an ice chest and saved for a test which was made a week later. In this, twelve instead of ten dilutions were used. It was found, however, that the additional dilutions were not necessary, because the titres of the antisera had gone down during the week's storage.

### Discussion

The results of the experiments are recorded in tables I-V. The results of the tests with samples of sera drawn before immunization of each animal used against suspensions of the organisms used in the tests with the antisera later on are not presented in tabular form. The set-ups were exact duplicates of the tests with the antiserum of the same animal against suspensions of the organisms used.

As pointed out in Part I, some spontaneous agglutination or settling out occurred in tests of the normal sera of the animals against suspensions of *B. carotovorus* 3a, *B. aroideae*, and *Bact. tumefaciens*. This, however, also occurred in the control suspensions in saline. Later, proper precautions were taken in preparing suspensions, as indicated in Part I, and settling out was avoided. It was con-

TABLE I\*  
ANTISERUM OF BACTERIUM CAMPESTRE AGAINST

SUSPENSION OF (ANTIGEN)	ANTIMAL	TUBES										CONTROL
		1	2	3	4	5	6	7	8	9	10	
<i>B. aroidae</i> .....	{ 87 154	++ .....	++ ++	- +	- -	- -	- -	- -	- -	- -	- -	- -
<i>B. carotovorus</i> 3a...	{ 87 154	- .....	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
<i>Bact. campestre</i> ....	{ 87 154	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	- -

\* In table I and all the following tables where the symbols are used, ++++ stands for complete clearing of the suspension with total deposition in a clump; +++ stands for complete clearing but only partial deposition in a clump, many clumps being in suspension; ++ stands for partial clearing with a small clump and some clumps in suspension; + stands for partial clearing with a very small clump and a few clumps in suspension; and - stands for no change in turbidity. All readings were made macroscopically as well as with a hand lens.

In control tests with the sera of these animals before immunization, all tests were negative excepting those against suspensions of *B. carotovorus* 3a and *B. aroidae*; these gave very slight agglutination or settling in all dilutions of sera, as well as in the saline control (see page 200).

TABLE II\*  
ANTISERUM OF BACTERIUM MEDICAGINIS VAR. PHASEOLICOLA AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CONTROL
		1	2	3	4	5	6	7	8	9	10	
B. aroideae. ....	164†	—	—	—	—	—	—	—	—	—	—	—
B. carotovorus 3a...	164	—	—	—	—	—	—	—	—	—	—	—
Bact. medicaginis var phaseolicola.....	164	+++	+++	++++	++++	++++	++++	++++	—	—	—	—

\* For data of the control tests with sera of the animals before immunization see footnote for table I.

† The second animal died after the last injection.

TABLE III\*  
ANTISERUM OF BACTERIUM TUMEFACIENS AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CONTROL
		1	2	3	4	5	6	7	8	9	10	
B. aroideae. ....	152 153	++ ++	++ ++	++ ++	— ++	— —	— —	— —	— —	— —	— —	— —
B. carotovorus 3a...	152 153	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
Bact. tumefaciens...	152 153	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	— —

\* In control tests with the sera of these animals before immunization, all tests gave very slight agglutination or settling in all dilutions of sera as well as in the saline control (see page 200).

cluded from the results that the sera of none of the animals before immunization agglutinated suspensions of *B. carotovorus* 3a or *B. aroideae*.

The antisera of *Bact. campestre*, *Bact. medicaginis* var. *phaseolicola*, and of *Bact. tumefaciens* failed to give agglutination in any dilutions (1-10 to 1-5120) with suspensions of *B. carotovorus* 3a. The antiserum of *Bact. medicaginis* var. *phaseolicola* also failed to give agglutination in any dilution with suspensions of *B. aroideae*. The antisera of *Bact. campestre* gave partial agglutination in dilutions 1-40 with suspensions of *B. aroideae*, and the antisera of *Bact. tumefaciens* gave partial agglutination in dilutions 1-80 against suspensions of *B. aroideae*.

The antisera of *B. aroideae* and of *B. carotovorus* 3a gave no agglutination against suspensions of *Bact. campestre* and *Bact. medicaginis* var. *phaseolicola*, but did give titres of 1-320 against suspensions of *Bact. tumefaciens*. Obviously, then, the agglutination test can be used to differentiate the soft-rot organisms *B. aroideae* and *B. carotovorus* from *Bact. campestre*, *Bact. medicaginis* var. *phaseolicola*, and *Bact. tumefaciens*.

The antiserum of *B. aroideae* (animal no. 173) tested against suspensions of the homologous organism gave complete agglutination through all dilutions (1-10 to 1-5120) in one test, and a titre of 1-10240 with complete agglutination in dilutions 1-10 to 1-1280 in the second test. The antiserum obtained from the second animal (no. 174) gave complete agglutination in all dilutions in the first test (1-10 to 1-5120), and a titre of 1-20480 with complete agglutination in dilutions 1-10 to 1-1280 in the second test. In addition, the antisera of *B. aroideae* also gave group agglutination against suspensions of *B. carotovorus* 3a. The antiserum (animal no. 173) tested against suspensions of *B. carotovorus* 3a gave a titre of 1-5120 with only partial agglutination in all dilutions in the first test, and a titre of only 1-160 in the second test; the other antiserum (animal no. 174) in the first test gave a titre of 1-80 with complete agglutination in dilutions 1-10 to 1-40, and in the second test gave the same titre but complete agglutination only in dilution 1-10. Apparently the antiserum of *B. aroideae* can be used to diagnose *B. aroideae*, and to differentiate *B. aroideae* from *B. carotovorus* 3a.



TABLE V\*  
ANTISERUM OF *B. CAROTOVORUS* 3A AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TEST	TUBES												CONTROL
			1	2	3	4	5	6	7	8	9	10	11	12	
<i>B. aroides</i> .....	66†	1	+++	+++	+++	++	++	++	+	+	—	—	—	—	
		2	+++	+++	++	+	+	+	—	—	—	—	—	—	
<i>B. carotovorus</i> 3a....	66	1	+++	+++	+++	+++	+++	+++	+++	+++	++	—	—	—	
		2	+++	+++	+++	+++	+++	+++	+++	+++	++	—	—	—	
<i>Bact. campestre</i> .....	66	.....	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Bact. medicaginis</i> var. <i>phaseolicola</i> .....	66	.....	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Bact. tumefaciens</i> ...	66	.....	+++	+++	+++	+++	+++	+++	++	++	—	—	—	—	

\* In control tests with sera of these animals before immunization, the tests with *Bact. campestris* and *Bact. medicaginis* var. *phaseolicola* were negative; the tests with *B. aroides*, *B. carotovorus*, and *Bact. tumefaciens* gave slight agglutination or settling out in all dilutions of sera as well as in the saline control (see page 200).

† Injected intraperitoneally.

The antiserum of *B. carotovorus* 3a tested against suspensions of the homologous organism gave titres of 1-1280 and 1-2560 with complete agglutination in dilutions 1-10 to 1-80 (++++) in both sets of tests. It also gave group agglutination with suspensions of *B. aroideae*. Against suspensions of this organism it gave titres of 1-640 and 1-320 with complete agglutination (++++) in dilutions 1-10 to 1-20 in the first test, and in dilutions 1-10 and 1-20 in the second test. These tests indicate that the antiserum of *B. carotovorus* 3a lends itself to differentiation between *B. carotovorus* 3a and *B. aroideae*.

These results can be interpreted to indicate that, although *B. aroideae* contains some of the antigens which stimulate production of the same antibodies as *B. carotovorus* 3a, it also contains others that stimulate production of different antibodies from *B. carotovorus* 3a; therefore *B. aroideae* can be differentiated from *B. carotovorus* 3a on this basis. The results can be interpreted to indicate further that, while *B. carotovorus* 3a contains antigens which stimulate production of antibodies that react with suspensions of *B. aroideae*, it apparently contains others to some extent that stimulate production of different antibodies from *B. aroideae*; therefore it can be differentiated from *B. aroideae* on this basis.

### Conclusions

1. The agglutination test can be used to differentiate *Bacillus aroideae* and *B. carotovorus* of the soft-rot group from *Bacterium campestre* of the *Bact. campestre*-*Bact. phaseoli* group, from *Bact. medicaginis* var. *phaseolicola*, and from *Bact. tumefaciens*.
2. Serologically, *B. aroideae* and *B. carotovorus* are distinct although closely related.
3. Maintenance of *B. aroideae* and *B. carotovorus* as distinct species is justified serologically.

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# ABSCISSION IN COTTON FLOWERS<sup>1</sup>

CHARLES P. DUTT

(WITH SEVEN FIGURES)

The cotton plant is sensitive to sudden changes in its environment, and this sensitiveness is manifested by modified growth, coloration of the bark and leaf, and the shedding of leaves and flowers. There may also be other manifestations. Abscission has been described as self-pruning of unnecessary parts of the plant, but considered from the growers' point of view, any practice by which abscission in the cotton plant could be prevented or minimized and by which the bolls could be brought to maturity would be an advantage. The causes in detail and the mechanism of abscission are little understood. Some work has been done to determine the exact changes that take place, but there is not sufficient evidence for some of the explanations that have been advanced.

Since there is some non-uniformity in the meaning of terms used by various investigators who have worked with abscission, the terms used in this paper (which are those upon which the majority of investigators agree) are briefly defined as follows: Abscission is the detachment of an organ by the separation of actively living cells. The separation layer is the layer of cells, the components of which separate at time of abscission. The separation cells or absciss cells are cells that make up the separation layer. The abscission zone is the general region in which abscission occurs.

## Historical review

VON MOHL (17) was the first to announce that a definite separation layer is formed at the base of the petiole before the fall of the leaf. He pointed out that abscission always occurs by the separation of the cells from one another while their walls are still intact. WIESNER (21) confirmed this observation, and formulated the theory that the dissolution of the intercellular substance of the cells of the sep-

<sup>1</sup> Contribution from Division of Agronomy, University of California.

aration layer is caused by the development of organic acid in the leaves. MANGIN (16) discovered the pectic nature of the middle lamella of cell walls in plants, and since this discovery abscission has been referred to as a dissolution of the middle lamella.

The anatomical workers disagree as to the amount of cell wall altered during abscission. LEE (10) reported the disappearance of the middle lamella in foliar abscission. HANNIG (6) reported the same condition in the abscission of flowers of *Mirabilis jalapa*. TISSON (20) and LEE (10) reported that the secondary layer of the cell wall also was altered to some extent, only the thin tertiary membrane lining the cell cavity remaining. LLOYD (12) found the same condition in the abscission of cotton bolls and flowers of *Mirabilis jalapa*. SAMPSON (19) and HODGSON (7) suggested that enzymic activity may be an important factor in abscission.

BALLS (2) reported that the abscission zone of cotton flowers is marked by an external groove. He described the abscission layer as a layer of meristematic cells, one cell thick. The divisions begin in the cortex and extend inward; the dividing wall between the daughter cells splits immediately along its middle lamella. The daughter cells which are left on the face of the scars, after the pedicel has been broken away, bulge outward and form a callus. LLOYD (15) found that in young cotton bolls only one tier of cells is involved, while in old tissues, such as the internode, there are ten to twelve tiers of cells involved, and these show evidence of physiological activity. He stated that the greatest physiological activity occurs in one or two tiers of cells which occupy the upper limit of the abscission zone and constitute the separation layer. The cell walls are altered most in the separation layer. This alteration proceeds so far as to digest completely a part of the primary and secondary walls.

ATKINSON (1) held the view that abscission is a "purely physiological trouble." He stated that the immediate cause in cotton flowers is the interference of a saturated soil with root activity. BALLS (2) stated that abscission is due largely to environmental influence and not exclusively to soil water conditions, although the position of the water table has been shown to have a major influence in the irrigated regions of Egypt. He induced boll abscission by asphyxiating the root system, by immersion in water, and by root

pruning. BALLS found a rapid loss of water from bolls when removed from the plant, and suggested that there might be an actual net loss of water during the day if the roots were unable to supply the amount needed. A rising water table may reduce the roots, and may produce the same condition. The work of LIVINGSTON and BROWN (11), and of LLOYD (14) on the deficit of water in leaves, and BALLS' own observations on the cessation of growth in sunshine favor this view. BARANETSKY (3), working on the daily periodicity of growth, found that plants grow more slowly by day than by night; so it appears that light, as well as a deficit of water, exerts a retarding influence upon growth.

BALLS noted that wilting, or even a water deficit sufficient to lead to the closure of stomata, would result in the raising of the internal temperature, which might serve as a stimulus to abscission, especially when the change is sudden. He states: "we are unable to form a clear conception of the chain which extends from the severed root to the absciss layer." He is almost certain, however, that the main factor, if not the only one, is the water content of the plant. The absciss layer is the name given by BALLS to the separation layer.

BALLS, and LLOYD (15), found the transpiration rate of cotton leaves to be very high. BALLS found a loss of 1.8 gm. per 100 sq. cm. per hour, and LLOYD found the highest loss to be 5.25 gm. per 100 sq. cm. per hour. This indicates a heavy draft upon the water supply of the plant. LLOYD found that the water deficit limited growth; that the depletion of soil moisture was the cause of the gradually increased shedding as the season progressed; and that such shedding could be offset by irrigation.

The evidence obtained on the cotton plant by BALLS in Egypt and LLOYD in America strongly indicates that lack of water constitutes a stimulus leading to floral abscission. The work of PRESCOTT (18), on the other hand, shows that heavy irrigation may cause increased shedding. He found that when plants were given excessive amounts of water all the flowers were shed. As the amount of water was reduced the shedding was also decreased. Under "heavy," "medium," and "light" irrigations 85.4, 60.8, and 59.0 per cent of the flowers were shed respectively. With very light irrigation the shedding was 57.3 per cent. When a small amount of water was applied,

not only was the shedding reduced but the total number of flowers per plant was also greatly reduced. PRESCOTT'S work does not harmonize with LLOYD'S theory that shedding may be offset by irrigation.

HAMMOND (5) reported a very high rate of shedding under normal field conditions. The data are reproduced in table I, the monthly totals being summarized from the daily counts.

LLOYD (15) found that the time required for abscission varies with the age of the flowers and bolls. He found that squares and

TABLE I  
NORMAL SHEDDING IN COTTON

	DATE OF EMERGENCE			
	April	May	June	July
Number of plants.....	7	10	2	1
Forms appeared.....	1700	2586	154	60
Forms died.....	1231	1819	106	24
Forms bloomed.....	469	667	48	36
Blooms and bolls dropping:....	163	199	12	19
Bolls maturing.....	306	468	36	7
Per cent maturing.....	18	18	23	11

flower buds were the most susceptible to shedding. As the bolls become older they are less likely to be abscised. The bolls do not shed after the tenth day, unless wounded by insects or mechanical means. This investigator found that shedding was very rapid in young squares or unopened flowers. The abscission may occur before or after flowering, but during anthesis there occurs a partial or complete inhibition of the abscission process. He found the usual period for abscission in squares to be 48 hours. LLOYD was able to cause 100 per cent abscission of flowers by preventing pollination. He found the abscission period to be extended with the increasing age of the bolls, the maximum length of time being 6 days or less.

LLOYD (15) found that prevention of pollination by the removal of stamens was followed by total shedding of the young bolls in 48 hours. HODGSON, working with LLOYD and JIVANNA RAO (8), found that if pollen grains were placed in water, they burst immediately and were rendered impotent for fertilization. LLOYD points out that

rain on open flowers undoubtedly causes the pollen grains to burst, or otherwise injures the flower, and that the damage is lessened where the flowers are protected by overhanging leaves. The time of rainfall is undoubtedly very important; a light shower when the pollen is being discharged might do more damage than a heavy rain at any other time.

KENDALL (9), working on abscission of fruits and flowers in the Solanaceae, was unable to induce abscission with narcotic vapors, injury to floral organs, sudden rise in temperature, lack of pollination, or changes in soil conditions. The last he recognizes as an indirect cause.

GOODSPEED and KENDALL (4) report that more cells are involved in abscission in the  $F_1$  generation of *Nicotiana* hybrids made with *Nicotiana sylvestris* than in the parents. This indicates that abscission is involved in Mendelian heredity.

### Technique

The writer has studied the abscission zone and separation layer in pedicels of cotton. Microscopic examination of fresh freehand sections was supplemented with preparations made by the paraffin method. The freehand sections were mounted in water without killing. The microchemical tests were made on somewhat thick freehand sections; thin sections were found to be unsatisfactory. The material used for sectioning in paraffin was killed in formalin-alcohol, absolute alcohol, and various concentrations of the chromo-acetic series. Weak chromo-acetic solution (Shaffner's formula) gave best results.

Longitudinal sections were supplemented by cross-sections through the base of the pedicel. The sections were cut 10  $\mu$  in thickness, and stained with safranin and Delafield's haematoxylin.

### Abscission types

The approximate position of abscission is usually marked by a faint external groove (figs. 1-3). This groove is present in mature flower bud pedicels, but is faint in very young ones. The groove is formed as a result of the difference in the rate of growth of the corti-

cal cells. In general there are two types of grooves; it may be ovate or somewhat rounded, extending about the same distance above and below the point of attachment (fig. 1), or it may be ovate, extending toward the lower node and having a considerable extension on the lower side. In extreme cases it may extend the entire length of the internode, and result in the removal of a strip of cortex (fig 3).

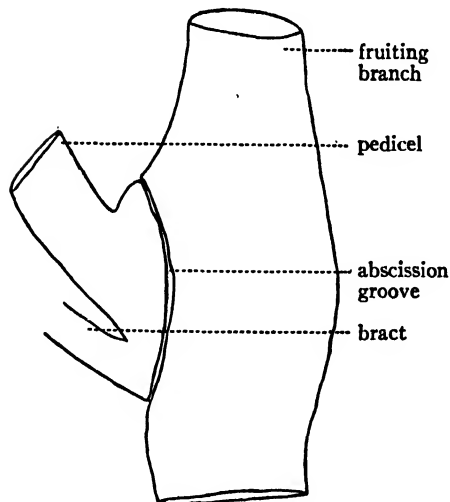


FIG. 1

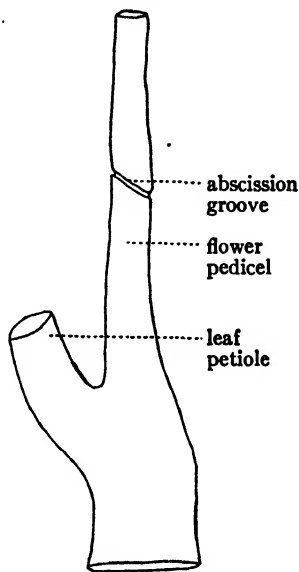


FIG. 2

FIGS. 1, 2.—Fig. 1, side view of circular abscission. Fig. 2, type of abscission in which only portion of pedicel is abscised.

Observations show that when the groove is ovate or somewhat rounded abscission is complete (figs. 4, 6). In this type the groove is continuous through pedicel and petiole. The position of the groove is in the internode at the base of the pedicel, slightly back of the juncture of the leaf petiole. When the groove occurs near the center of the internode, the leaf petiole is not involved (fig. 2). In this type the abscission is complete, the abscised part falls off very readily unless it is old, and the vascular tissues have become well developed. When the groove extends down the stem for a considerable distance the excised part often persists and dies in place, drying slowly. In attached ones there remains a slight connection of the vascular system, which prevents rapid drying.

### Abscission zone

The external groove indicates in a general way the position of the abscission zone, although the latter does not always cross in the plane indicated by the external groove. It may develop to either side of it or along its upper edge, but in the majority of cases the abscission zone coincides and lies directly beneath it. When young pedicels are examined there is no

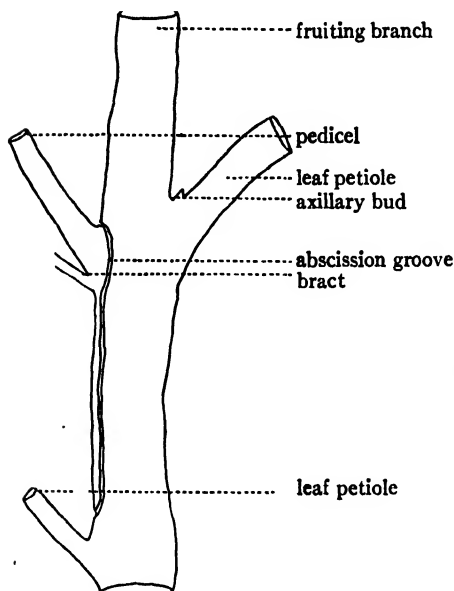


FIG. 3

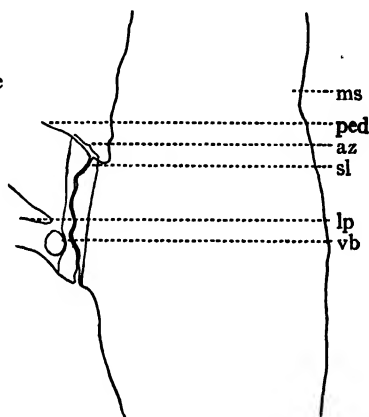


FIG. 4

FIGS. 3, 4.—Fig. 3, side view of downward projecting abscission. Fig. 4, diagram showing various parts of abscission: *ms*, main stem; *ped*, flower pedicel; *az*, abscission zone; *sl*, separation layer; *lp*, leaf petiole; *vb*, vascular bundle (note that abscission zone and separation layer are deflected from vascular bundle).

apparent differentiation of any anatomical structure, hence the abscission zone does not appear to be pre-formed.

The abscission zone involves ten to twelve tiers of cells (fig. 5). The cell walls of the abscission zone swell and may become twice the normal thickness (fig. 6), and their power to take up and hold stains is reduced.

Contrary to the findings of BALLS, there is no evidence of cell division in the abscission zone at any time during the process.

Actual separation usually involves one or two rows of cells (fig.

6). The greatest physiological activity occurs in this layer, the tissue disintegrating by the dissolution of the cell walls. Separation does not occur straight across the pedicel; in most cases it follows a zigzag

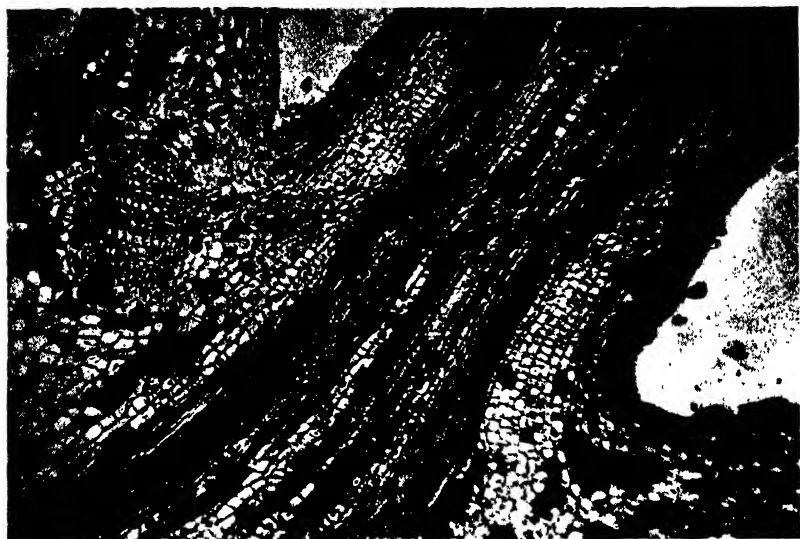


FIG. 5.—Early stage of abscission zone; petiole at upper left, pedicel at upper right

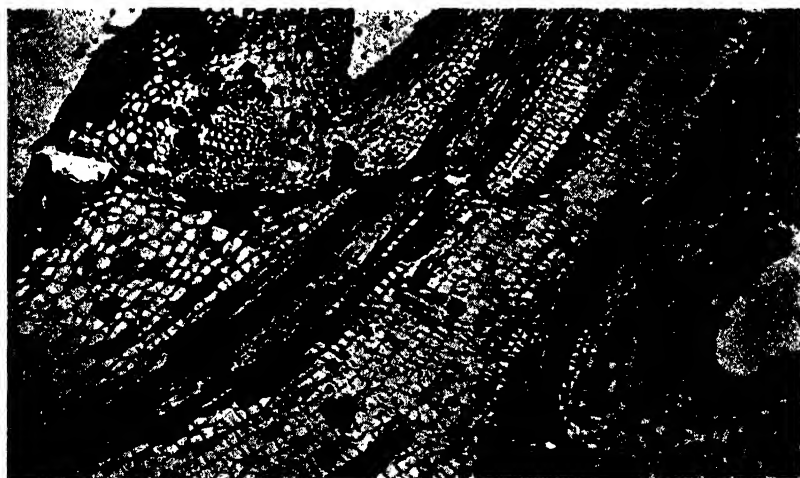


FIG. 6.—Separation layer with pockets; petiole at upper left, pedicel at upper right



course (fig. 4) and often contains pockets (fig. 6). These pockets contain single cells or groups of cells that have resisted chemical alteration.

### Abscission process

Before abscission is initiated the cells of the abscission zone are well supplied with starch. The starch here is far in excess of the amount present in the tissues on either side. This relationship is reversed as soon as abscission begins, that is, the starch is then more abundant in the surrounding tissues than in the abscission zone. By the time abscission starts, a large amount of the starch is either used up or removed by translocation, although some of it remains until the separation layer becomes well defined. The rapid chemical changes which occur in the cell walls make doubtful the removal of starch from the cells by translocation. The starch is most likely used by these cells as a source of energy.

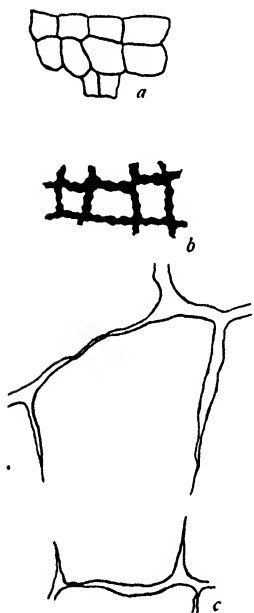


FIG. 7.—Cells from separation layer showing changes in cell walls: *a*, normal cells; *b*, thickened cell walls; *c*, cell showing dissolution of wall.

If thick longitudinal sections through the abscission zone are stained with chlor-iodide of zinc and potassium iodide-iodine a purple band is formed, delimiting the abscission zone. Examination of sections at subsequent stages shows that the starch content in the cells of the abscission zone decreases as abscission progresses.

The second indication of a change in the abscission zone is the thickening of the cell walls, which become about twice the original thickness (fig. 6).

The earliest indication of chemical alteration is the decreased power of the cell wall to take and hold stains. Thick sections stained with iodine previous to abscission show the cell walls distinctly colored, but if stained shortly after the beginning of abscission the cell walls show a much lighter color than adjacent cells outside of the

abscission zone. This indicates a chemical change of cellulose to hydrocellulose.

If thick sections are boiled in weak hydrochloric acid (about 4 per cent) for one minute and then treated with strong potassium iodide-iodine, the cell walls of the abscission zone show a faint greenish hue. If the sections are washed gradually, the yellow of the iodine disappears and the walls show a pale blue, indicating hydrocellulose. This pale blue fades away if washing is continued, but the color does not fade in the cells adjacent to the abscission zone. The fading of the stain shows the decreased power in holding it, since the color intensifies as the distance from the abscission zone increases. Thick sections, stained in dilute methylene blue overnight, show that the cells of the abscission zone do not hold the stain as do the neighboring cells. The decreased power of the cell walls to take up and hold stains indicates a chemical alteration in them.

Microchemical examination shows that the cell walls are composed of cellulose before abscission sets in. If sections are treated with 75 per cent sulphuric acid, then stained with iodine, a blue color develops in the cell walls, indicating cellulose. If sections are stained with methylene blue after the process of abscission is under way, a violet color develops in the center of the cell wall, indicating the presence of pectic substances.

If thick sections are treated with 5 per cent sulphuric acid, large needle crystals of calcium sulphate are formed in the cells. These tests show calcium to be present in all cells before abscission, but as abscission nears completion it decreases. When abscission is completed the calcium disappears in the abscission cells, while it is plentiful in adjacent cells.

A partial chemical alteration involves a zone of ten to twelve rows of cells (fig. 5). The greatest degree of alteration takes place in one or two rows of cells near the boll side of the abscission zone. The cell walls of these rows of cells are completely altered and dissolved. This produces a complete separation (fig. 6), but does not necessarily occur directly across the pedicel. The separation layer first forms in the innermost layer of the cortex, and is propagated outward toward the epidermis. The abscission zone forms in the pith, separately but simultaneously. The abscission is completely

inhibited in the tracheae of the vascular system. The tracheal tubes show evidence of being pulled apart mechanically.

### Relation of soil moisture to water content of cotton

Study of the abscission problem raised the question of the relation of the moisture content of the soil to the moisture content of different parts of the cotton plant, consequently samples of different parts of the plant were taken on four different dates, and at the

TABLE II  
MOISTURE CONTENT OF COTTON TISSUES AND SOIL

	DATE OF SAMPLE			
	August 19	August 23	August 31	September 6
	Percentage moisture content of tissues			
Flower.....	82.86	82.22	81.27	80.22
Young bolls.....	85.79	85.22	85.34	82.22
Mature bolls.....	85.62	81.61	82.73	80.20
Upper leaves.....	76.31	74.06	73.17	73.08
Lower leaves.....	75.15	74.90	73.84	72.75
Upper stem.....	83.52	71.86	82.10	68.48
Middle stem.....	75.43	71.58	70.51	65.63
Lower stem.....	70.98	65.87	67.05	64.01
Root.....	67.95	69.12	71.99	68.31
Pedicle normal.....	82.49	81.01	78.09	77.05
Pedicle abscising.....	74.06	73.09	67.02	64.46
	Percentage moisture content of soil			
First foot.....	20.33	15.65	13.61	11.38
Second foot.....	20.65	15.40	15.00	12.55
Third foot.....	20.12	19.53	13.66	11.84
Fourth foot.....	19.32	18.13	16.63	11.58
Average of 4 feet.....	20.10	17.17	14.72	11.84

same time soil samples were taken under the plant sampled. All samples were in duplicate and oven dried at 100° C. The soil was irrigated on August 18. The results are shown in table II.

The figures in the table bring out one striking relationship, namely, that in general the water content of the cotton plant decreases as the water content of the soil decreases, showing the direct effect of soil moisture on the water content of the cotton plant. It should be noted that young tissue, such as flowers, leaves, and young bolls decline only slightly in moisture content as time from irrigation in-

creases; the moisture in roots even increased over the three-week period. The moisture content of both the normal and abscising pedicel declined rapidly.

### Summary

1. Abscission of the cotton flower usually occurs at or near the base of the internode, but sometimes in the middle.

2. A groove or surface depression generally indicates the position of the abscission zone.

3. In very young material there are no apparent histological differences setting off the abscission zone from the tissues on either side.

4. The abscission zone consists of ten to twelve rows of cells.

5. The separation layer is located near the upper or distal region of the abscission zone, and consists of one or two rows of cells.

6. The separation layer is formed by the chemical dissolution of the cell walls.

7. There is no evidence of cell division in the abscission zone of the cotton flower.

8. All the tissues across the pedicel except the tracheal tubes function in abscission.

9. The first visible indication of abscission is the swelling of the cell walls, followed by their partial solution.

10. Prior to abscission, large quantities of starch are stored in the abscission zone, which later disappears during the development of the abscission zone.

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## STUDIES IN THE GENUS MENTHA

### 15. ACTION OF PEPPERMINT PEROXIDASE ON MENTHOL

SAMUEL M. GORDON<sup>\*</sup>

In a previous paper (6) on the quantitative relations of catalase and peroxidase in the growing peppermint plant, it was shown that both of these reached a maximum at the flowering period. Just past the flowering period these enzymes took a decided drop. It was further shown that the florets showed little oxidizing capacity in terms of catalase and peroxidase values. Whether the increase in the oxidizing enzymes in the leaf at the flowering period is related to the oxidation of menthol is difficult to decide, in view of the contradictory analytical values of CHARABOT (3) and KLEBER (7). On the one hand, CHARABOT claimed that the content of menthone (oxidation product of menthol) increased with the vegetative process, and at the expense of the previously formed menthol. KLEBER, however, showed that the oil derived from younger plants was richer in menthone. The former worker also claimed that when the plants were deprived of their inflorescences the oxidation of menthol was insignificant. The results of CHARABOT are harmonized with the oxidase values by assuming a transportation of the menthone from the leaf to the floret. It has not definitely been ascertained, however, that the plant or an extract of it can bring about this oxidation. If an extract or some portion of the plant were available, which could oxidize the alcohol to the ketone, such an assumption could safely be assumed. BROOKS (2) attempted to carry out such an oxidation but obtained a negative result. The extract used by him yielded the usual color test for oxidases.

Once more taking CHARABOT's analytical results as a point of departure, it will be recalled that his data showed an increase in the amount of unsaturated hydrocarbons present at the period of inflorescence, and he states that at this time an increased oxygen absorption is taking place. This increased content of hydrocarbon takes on

<sup>\*</sup> National Research Council Fellow in Biological Sciences.



was passed into the system, they were dried as far as possible on the force filter, and then in a vacuum desiccator over sulphuric acid. The enzyme preparation was not further purified than described later (9).

The extracts were obtained from sturdy plants at the height of the flowering period from the plat mentioned in a previous report (6). Twenty gm. of the fresh leaves was used as the source of the enzyme in most of the experiments. As soon as the plant material reached the laboratory, the leaves were stripped from the stems, shredded with scissors, ground with purified sea sand, and extracted with 200 cc. of water and allowed to digest for two hours. The filtered extract was treated with an equal volume of alcohol, filtered, and again taken up in 100 cc. of water and precipitated with alcohol.

All of the oxidase preparations yielded well defined reactions with the usual color reagents, including hydrothymoquinone.

EXPERIMENT I.—(a) 25 cc.  $\alpha$ -pinene saturated with wet oxygen, 2 gm. menthol; (b) same as (a) but  $\beta$ -pinene used; (c) same as (a) but d-limonene used. The mixtures of menthol and oxygenated hydrocarbons were placed in 500 cc. round-bottom flasks, and set in direct sunlight for thirty days. The hydrocarbons were removed by distilling in vacuum and the residue tested for menthone by means of semicarbazide hydrochloride in the usual way. In all three cases a negative test was obtained, and the menthol was recovered almost quantitatively by steam distillation.

EXPERIMENT II.—The same result was obtained with hydrocarbons saturated with wet oxygen.

EXPERIMENT III.—Experiments I and II were repeated, but oxygen was passed into the mixtures for approximately eight hours a day for thirty days. No oxidation took place and the menthol was again recovered quantitatively.

EXPERIMENT IV.—A sample of oxidase obtained from 50 gm. of leaves and 2 gm. of menthol was allowed to stand at room temperature (about 23°) for fifteen days. A menthone test was negative and the menthol was recovered.

EXPERIMENT V.—Experiments I, II, and III were repeated but with the addition of an oxidase preparation from 20 gm. of leaves. The results were the same as before.



EXPERIMENT VI.—The test was carried out as for Experiment V, but oxygen passed in. The results were negative for menthone in all six cases, the menthol being almost totally recovered.

EXPERIMENT VII.—The protocol was carried out as the previous experiment, but shaken continuously for fifteen days. Identical results as previously mentioned were obtained.

EXPERIMENT VIII.—The previous experiments were modified by using ferrous sulphate as an additional catalyst, since it has been shown that ferrous salts acting as a catalyst on hydrogen peroxide may effect certain oxidations (4). The experimental mixture was made up as follows:

- 25 cc.  $\alpha$ -pinene saturated with wet oxygen
- 2 gm. menthol
- oxidase obtained from 20 gm. leaves
- 0.5 gm. of ferrous sulphate in 10 cc. of water.

When shaken a stable emulsion formed. The reaction flask was shaken continuously for two weeks and oxygen passed into the mixture for the duration of the experiment. The terpene components were recovered in the usual way and tested for menthone. A negative result was obtained.

EXPERIMENT IX.—The mixture was made up of 25 cc. of  $\alpha$ -pinene saturated with oxygen, 2 gm. of menthol, and oxidase from 20 gm. leaves in 100 cc. of water. Oxygen was passed in for fifteen days and continuously shaken. The reaction mixture was worked up in the usual way. A negative test for menthone was obtained and the menthol recovered quantitatively.

As a final check on the absence of menthone, the combined menthol fractions were tested for menthone with semicarbazide hydrochloride in the usual way. No menthone semicarbazone was obtained.

These results conclusively show that the oxidizing enzymes, isolated from *Mentha piperita* L., alone or in the presence of so-called terpene peroxides, apparently simulating conditions in the plant, do not possess the power of oxidizing menthol to menthone. While quantitative results show the presence of considerable amounts of oxidizing enzymes in the plant, they must be considered as not related to a direct oxidation of menthol. Several experiments have

been completed in this laboratory, which serve to show that the menthol may be produced from menthone by a modified Cannizzaro reaction. In this case a reduction instead of an oxidation is involved, and is in agreement with the newer theories of WIELAND. These results will form the subject of a separate report.<sup>2</sup>

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<sup>2</sup> Since submitting this manuscript to the editor, these results have been reported in Jour. Biol. Chem. 75: 163. 1927.

## BRIEFER ARTICLES

### A CHLORINATION METHOD FOR MACERATING WOODY TISSUES

(WITH ONE FIGURE)



During recent investigations into the chemical nature of the cell walls of plants,<sup>1</sup> thin transverse sections of wood were exposed to the action of chlorine water followed by hot sodium sulphite solution to dissolve out the lignin. By this treatment the middle lamella was removed, leaving a residue of disorganized cells. This general reaction has been employed by chemists for some time in the preparation of wood cellulose, but in so far as the writer is aware, has received scant attention among anatomists. The method seems well adapted to fiber macerations of woody tissues.

Several woods were macerated by this method, which is apparently capable of yielding material in which the structural details of the various cells are faithfully preserved. Fig. 1 is a photomicrograph of a vessel segment of *Magnolia* isolated by the chlorine-sodium sulphite reaction. Details of structure are likewise preserved with equal clearness in parenchymatous elements, and excellent mounts can be made which seem to surpass those obtainable with the more drastic maceration methods now in general use. It seems probable as well that this procedure will adapt itself to studies of phloem tissues, since the writer was able to obtain seive tubes in fairly good condition from a maceration of *Populus* bark.

The method is as follows: (1) split out material match-stick size; (2) boil to expel air; (3) immerse in strong chlorine water for 2 hours; (4) wash; (5) immerse in hot 3 per cent sodium sulphite solution 15 minutes; (6) wash; (7) repeat the process from no.

FIG. 1.—Photomicrograph of vessel segment of *Magnolia*;  $\times 120$ .

<sup>1</sup> The chemical nature of the middle lamella. Tech. Publ. 21. N.Y. State Col. Forestry, Syracuse. 1927.

3 until, upon mild shaking, sufficient material separates for the purpose in view. When the red color first obtained upon addition of the sodium sulphite fails to reappear in this reagent after several chlorinations, the wood should be completely delignified.

Acknowledgments are due Dr. H. P. BROWN for helpful suggestions in preparing the manuscript.—WILLIAM M. HARLOW, *Department of Wood Technology, N.Y. State College of Forestry, Syracuse, New York.*

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# CURRENT LITERATURE

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## BOOK REVIEWS

### Soil conditions and plant growth

This valuable book by RUSSELL<sup>1</sup> has maintained its leadership in the field of knowledge covered, for fifteen years. It has now reached its fifth edition, and is the best presentation of the influence of soil conditions on the development of plants in the English language. The book needs no description, since it is so widely known and read. There are some changes in chapter headings, and in order of presentation they may be noted. Chapter IV, formerly the Colloidal Properties of the Soil, is now Chemical and Physical Relationships of the Soil. Chapter VII has been made chapter VI in the new edition, and chapter VI becomes chapter VII with the title Biotic Conditions in the Soil. The final chapter is entitled Methods of Soil Examination instead of Soil Analysis and its Interpretation. Appendix II of the former edition has been incorporated into the text.

RUSSELL is doing a splendid service for all students of the soil and plants in keeping this book up to date. The advances have been more rapid during the last six years than at any time in history, and every chapter has been amended to present the newer aspects of the soil relations. In commenting on the rapid advances made recently in the chemical investigation of the soil, as compared with the inactivity of this subject a few years ago, the author makes a statement that should not be forgotten by those who control the purse strings for research activity. He says: "Once again it has been shown that progress in applied science is limited by the state of knowledge of pure science, and that the surest way of solving technical problems is to investigate the underlying principles and causes." The book deserves a place in every library. The only criticism that one feels like making is that a book to be used as much as this one will be deserves to have a more durable binding.—C. A. SHULL.

### Colorado plant life

The movement toward the popularization of science has resulted in the production of a series of non-technical books of the vegetation of certain limited areas. Among the better examples of this type of volume is RAMALEY's description of the vegetation of Colorado.<sup>2</sup> This is not a local flora, it is much more, as may be seen from the following selection from its chapter headings: Plant

<sup>1</sup> RUSSELL, SIR E. J., Soil conditions and plant growth. 5th ed. 8vo. pp. viii+516. London: Longmans Green and Co. 1927.

<sup>2</sup> RAMALEY, FRANCIS, Colorado plant life. pp. 299. figs. 133. 3 colored plates. University of Colorado, Boulder. 1927. \$2.00.

sociology; Life zones and altitude; Mountain parks; Mountain lakes; Mesas and foothills; Plants of the true mountains; Forests and forest trees; Flora of Colorado. In these chapters there is enough of the principles of plant ecology to interest and instruct the amateur naturalist, together with enough of actual contribution to knowledge to make the volume worth while for the professional botanist. For the former there are numerous illustrations, some of them well colored, and for the latter there are classifications of plant communities and critical notes on the associations of the various zones. The subalpine associations of the mountain parks, the stream, and the lake side have been carefully studied by the author, and the reader will profit from this rich experience.

The volume is one in a series of semi-centennial publications of the University of Colorado, and is well suited to extend the influence of the university far beyond its walls.—G. D. FULLER.

#### NOTES FOR STUDENTS

Origin and development of plant sociology.—In 1922 PAVILLARD, in an article entitled *Cinque ans de phytosociologie*, and previously noted in this journal,<sup>3</sup> ascribed the first recognition of this phase of plant science to HARPER.<sup>4</sup> This is perhaps correct, in so far as America and western Europe are concerned, but in eastern Europe it seems to have developed much earlier, and to have been recognized by PACZOSKI in 1896, who used the term in a sense practically identical with that accepted today. Later PACZOSKI<sup>5</sup> points out the heterogeneous structure of plant communities, which are built up of different species, while human society is relatively homogeneous. He stresses the influence of the plant population by pointing out that in spite of changes in environmental conditions, such as the draining of swamps, certain plant communities frequently persist for a long time, but changes come quickly as the result of the introduction of new elements in the plant population. He also states: "Phytosociology, as I conceive it, has to do with plant communities in a manner different from any other department of botany. The limits of the distribution of plants are rarely set by climatic factors. Often a plant does not grow in a given region because it is not able to compete with indigenous plants. This is so regardless of the fact that the climate is not unfavorable for it if it were growing alone (without competition). Phytosociology is the basis on which phytogeography has to be built, if the latter is to be a real science."

Almost simultaneously, according to ALECHIN,<sup>6</sup> the Russian scientists ac-

<sup>3</sup> BOT. GAZ. 76:110-112. 1923.

<sup>4</sup> HARPER, R. M., The new science of plant sociology. Sci. Monthly 4:456-460. 1917.

<sup>5</sup> PACZOSKI, JOSEPH, Einige historische Angaben aus dem Gebiet der Phytosociologie. Bot. Notiser 1925:320-324. 1925.

<sup>6</sup> ALECHIN, W. W., Wann und wo ist die Phytosociologie entstanden? Bot. Notiser 1924:189-194. 1924.

cepted this phase of plant science, and KRYLOFF of the University of Tomsk, in 1898, wrote "The social relations of plants are being investigated. This branch of science is still young and may be called phytosociology." Further, in an introduction to a work on plant communities (Petrograd, 1915), SUKATSCHEW wrote "We must assume that phytosociology is now well established. The facts observed in a plant society resemble so closely those of human society that phytosociology best expresses this special branch of botanical science."

To these initial concepts may be added that of PAVILLARD and others, that phytosociology is the phase of plant science that endeavors to analyze vegetation inductively, by directing attention to the vegetation itself, rather than to the factors of the habitat. The most important unit is the association, and it aims to recognize and characterize all associations, designating them according to their characteristic or constant species. It has developed for itself a rather specialized terminology, as seen in the appearance at rather close intervals of two editions of a descriptive vocabulary.<sup>7</sup>

The two principal centers of its development have been termed respectively the Upsala and the Zurich-Montpellier Schools. Among the leaders of the former are DU RIETZ, GAMS, and ROMELL, while PAVILLARD, BRAUN-BLANQUET, and RÜBEL are prominent representatives of the latter.

In America sociological studies have not been very definitely separated from other phases of plant ecology. There have been, however, some investigations of plant populations, and among them three in which statistical methods have been applied to the vegetation of Michigan. GLEASON<sup>8</sup> has examined five virgin areas of maple-beech forest by means of strip count for the larger trees, and quadrat studies for the species of smaller size. The results of the census indicate a population of seventy-nine species, of which five trees, four shrubs, and fourteen herbs have a sufficiently high degree of frequency to be regarded as "characteristic species." *Acer saccharum* dominates the forest, with *Fagus grandifolia*, *Betula lutea*, *Ulmus americana*, and *Tilia americana* as important co-dominants. *Tsuga canadensis* is regarded as a relic of an earlier occupation of the land by coniferous forest. The deciduous forest is thought to be still young historically, and to have attained its full ecological dominance for only some three centuries.

A more extensive study of this association, with data on the percentage frequencies of the tree species in different portions of southern Michigan, has been made by QUICK.<sup>9</sup> Differences in the secondary species in different portions of the area are emphasized, and much importance is attached to historical factors

<sup>7</sup> BRAUN-BLANQUET, J., and PAVILLARD, J., *Vocabulaire de sociologie végétale*. 2d ed. p. 22. Montpellier. 1925.

<sup>8</sup> GLEASON, H. A., The structure of the maple beech association in northern Michigan. *Papers Mich. Acad. Sci. Arts & Letters* 4:285-296. 1924.

<sup>9</sup> QUICK, B. E., A comparative study of the distribution of the climax association in southern Michigan. *Papers Mich. Acad. Sci. Arts & Letters* 3:211-243. 1924.

and to competition. It is concluded that the beech-maple climax is capable, if left undisturbed, of occupying eventually all of the soils of the lower peninsula of Michigan, and of perpetuating itself on them.

KENOYER<sup>10</sup> has applied the methods of JACCARD and RAUNKIAER to a large number of plant associations in southwestern Michigan, determining the percentage of frequency occurrence for the individual species. More than 1000 determinations were made, and the results agree with those of RAUNKIAER in showing that species with lower frequencies are most numerous. When the species were arranged in five classes, on the basis of their frequencies, ranging from the least to the most abundant, the relative percentages of the number of species in the different classes were 70, 12, 6, 4, 7; in other words, the number of species in the least abundant class was ten times that in the most abundant. It was also found that when the number of species is small they tend to be confined to the first and the fifth groups; the same relationship was found to hold for the distribution of insects in grassland and for the distribution of micro-organisms in hay infusion; and the method seems excellent for determining whether or not an association is uniform.

Similar results were obtained by KOOPER,<sup>11</sup> in a study of the weed population in the fall on rice fields of Java. Since these weed communities agreed with natural vegetation in respect to the relative numbers of constant and infrequent species, RAUNKIAER's law of distribution of frequencies seems to be fairly universal in its application. This investigation by KOOPER makes use of weed communities as indicators of the suitability of the different soils of the Pasuruan plain for the growth of different varieties of sugar cane.

Still more recently, FREY<sup>12</sup> has reviewed this statistical method with particular emphasis on the graphic expression of the results of such studies. Since JACCARD was the first to emphasize the fact that the number of species of the most frequent plants was less than that of the less frequent, the curves representing these relationships are termed by FREY "JACCARD'S curves." Their use by RAUNKIAER, DURIEZ, and ROMELL is discussed, and several new possibilities of graphic representation are suggested. These are shown to be well suited to present such data as are now being published in investigations such as those conducted by the Zurich-Montpellier school of phytosociology. The application of GALTON's curve to the study of the association, by means of a "coefficient of significant species," seems to provide an exact method of testing the homogeneity of the plant population.

It is useless to attempt to enumerate the contributions of importance in

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<sup>10</sup> KENOYER, L. A., A study of Raunkiaer's law of frequency. *Ecology* 8:341-349. 1927.

<sup>11</sup> KOOPER, W. J. C., Sociological and ecological studies on the tropical weed vegetation of Pasuruan (island of Java). *Rec. Trav. Bot. Neerlandais* 24:x+252. 1927.

<sup>12</sup> FREY, ALB., Le graphique dans la phytosociologie. *Rev. Gen. Bot.* 39:533-546; 603-618. 1927.



this field, however, but a good list of the more important may be found in PAVILLARD's recent review of the situation.<sup>13</sup> In this attempt to evaluate the progress that has been made, he points out the limitations of such statistical and mathematical methods as the formula of ARRHENIUS, and agrees with GLEASON<sup>14</sup> and others that the distribution of species within an association follows the recognized laws of chance and probability. He further advocates the importance of plasticity and open mindedness on the part of investigators, and warns them against being tied down to formulae or to a special terminology. He commends a recent article by COOPER<sup>15</sup> which emphasizes the universality of change in vegetation.

COOPER aptly compares vegetation to a stream: "The vegetation of the earth is presented as a flowing braided stream. Its constituent elements branch and interweave, disappear, and reappear. Vegetation as we see it today is the advancing front of this stream, in which we discern more or less definite groupings that tend to repeat themselves in space, the advancing termini of the streamlet elements. There is a constant tendency toward merging of related elements and thus toward simplification into fewer and larger currents, which is balanced by a trend toward multiplication of the streamlet elements through forking. The vegetational stream is governed and directed by the interaction of factors residing in the constituent organisms and in their environment."—G. D. FULLER.

**Tertiary flora of Oregon.**—CHANEY<sup>16</sup> has published an important report of his investigation of the Tertiary flora of a region of eastern Oregon. After describing the general geologic features, he describes the known flora of three localities. Major attention is given to the fossil flora of Bridge Creek. The material consists mostly of well preserved leaf impressions. The presentation of the Bridge Creek flora includes descriptions of 44 species in 39 genera, representing 28 families. Most of the species are woody dicotyledons, mainly arborescent, only one fern, four conifers, and two monocotyledons representing these groups. Ten new species are described, and there is a full discussion of all the species. This region is especially favorable for an investigation of the ancient flora, since the material occurs in volcanic shales, which split readily along the bedding planes, making available an abundance of usable material.—J. M. C.

<sup>13</sup> PAVILLARD, J., Les tendances actuelles de la phytosociologie. Arch. Botan. 1:89-112. 1927.

<sup>14</sup> GLEASON, H. A., Species and area. Ecology 6:66-74. 1925.

<sup>15</sup> COOPER, W. S., The fundamentals of vegetational change. Ecology 7:391-413. 1926.

<sup>16</sup> CHANEY, RALPH W., Geology and palaeontology of the Crooked River Basin, with special reference to the Bridge Creek flora. Publ. 346, Carnegie Inst. Wash. pp. 45-138. pls. 8-20. 1927.

# THE BOTANICAL GAZETTE

May 1928

## STUDIES IN ONAGRACEAE<sup>1</sup>

### II. REVISION OF NORTH AMERICAN SPECIES OF SUBGENUS SPHAEROSTIGMA, GENUS OENOTHERA

PHILIP A. MUNZ

For this study there have been available almost 2000 sheets of herbarium material, which have come from the following collections, the abbreviation given in each case being that used in citing specimens: University of California (C), Herbarium of Frank Peirson of Pasadena (FP), Gray Herbarium of Harvard University (G), Missouri Botanical Garden, special material (M), Pomona College (P), Stanford University (S), United States National Herbarium, special material (US), University of Wyoming (W), Washington State College (WS). These herbaria have been visited or material has been borrowed from them. To those in charge of these collections, and especially to Dr. B. L. ROBINSON of the Gray Herbarium, I am much indebted for courtesies shown. I wish also to express appreciation to Dr. I. M. JOHNSTON of the Gray Herbarium for many kindnesses, and to Dr. H. M. HALL of the Carnegie Institution, to Director A. W. HILL of the Kew Botanical Garden, to Professor L. DIELS of Berlin, and to Dr. CARL CHRISTENSEN of Copenhagen for notes on and photographs of types.

In so far as this paper represents the results of a study of a part of a group on which more extensive work is under way, it may be considered as a preliminary revision. Up to the present time I have had access to insufficient South American material for any adequate

<sup>1</sup> No. 1 of this series appeared in *Amer. Jour. Bot.* 15: 223-240. 1928.

study of the species of that region, although in one or two cases I have seen fit to use South American specific names for North American plants. Until opportunity is offered for a more thorough study of the southern species I shall not be ready for a discussion of phylogeny. So far as I can make it do so, the key expresses my present idea of relationship among the species.

**Subgenus *Sphaerostigma*** (Seringe) JEPSON, Man. Calif. 680. 1925.

*Sphaerostigma* as sect., Seringe, in DC., Prodr. 3:46. 1828; as genus, F. & M., Ind. Sem. Hort. Petrop. 2:49. 1835. *Chamissonia* as genus, Link, Jahrb. Gewaechsk. 186. 1818. *Chamissonia* Endlicher, Gen. 1190, in syn., 1840. *Agassizia* as genus, Spach, Hist. Veg. Phan. 4:347. 1835. *Holostigma* as genus, Spach, Nouv. Ann. Mus. Paris (III) 4:332. 1835.

Calyx tube obconic to linear-funnelform; stamens subequal to distinctly unequal, and in 2 sets, all fertile; stigma capitate; capsule sessile or subsessile, cylindrical or narrowed toward tip, terete or quadrangular, usually curved or contorted; usually annual, with simple leaves; flowers white or yellow, often turning red or green on drying.

The application of Article 49 of the International Rules makes it necessary to use the name *Sphaerostigma* rather than *Chamissonia*.

#### KEY TO SPECIES

Flowers white (yellowish in *minor* and in 2 vars. of *decorticans*), often drying pinkish; borne in terminal spikes

Capsules cylindrical, terete, linear, not thickened in lower portion, scarcely if at all coiled, not noticeably attenuate at tip

Petals 5-7 mm. long, suborbicular; style exceeding corolla, 10-13 mm. long; calyx tube 4-6 mm. long; capsules refracted or spreading, occasionally coiled.—Deserts from S. Utah to Ariz. and S. California . . . . . 1. *O. refracta*

Petals 3 mm. long, spatulate; style shorter than corolla, 3.5-4 mm. long; calyx tube 2.5-3 mm. long; capsules divaricately spreading.—Deserts from W. Texas to S. Utah and S. California . . . . . 2. *O. chamaenerioides*

Capsules not strictly cylindrical, but somewhat enlarged near base and attenuate at tip

Mature capsules usually distinctly contorted and coiled, not merely bent and curved, quite slender, not subfusiform in shape (see also *O. decorticans* var. *desertorum*)

Flowers minute; petals 1-2 mm. long, narrowly obovate; style 1.5-3 mm. long; calyx tube 1-2 mm. long; longer filaments almost twice as long as shorter. . . . 3. *O. minor*

Flowers larger; petals 3.5-5 mm. long, orbicular-ovate; style 6-12 mm. long; calyx tube 3-8 mm. long; filaments subequal

Flowers and leaves arranged in spicate tufts at ends of naked prostrate branches or on short central stalk; capsules 10-12 mm. long, conspicuously quadrangular.—

Extreme W. Nevada. . . . . 4. *O. nevadensis*

Flowers and leaves continuous from base of stems, not in terminal tufts; capsules 14-23 mm. long, not conspicuously quadrangular. . . . . 5. *O. alyssoides*

Mature capsules merely curved or bent, not distinctly contorted nor coiled, subfusiform in shape

Leaves largely near base, glabrate, lance-ovate to oblanceolate; stems glabrous or glabrate, with epidermis exfoliating promptly; capsule 15-25 mm. long; seeds ash-colored and linear-obovoid. . . . . 6. *O. decorticans*

Leaves well distributed, glandular-pubescent to glandular-villous, ovate to oblong-ovate; stems glandular-pubescent to glandular-villous; epidermis exfoliating tardily, if at all; capsule 10-15 mm. long; seeds brownish, rhomboid-prismatic. . . . . 7. *O. boothii*

Flowers yellow, often drying greenish, borne in axils of foliage leaves

Capsules terete, cylindrical or subfusiform, but not quadrangular; leaves narrow, 1-4 mm. wide, usually linear-oblong

Plants with several naked, fine, often capillary stems, each bearing leafy inflorescence at tip; capsule subfusiform, almost straight, 5-8 mm. long.—E. Washington to E. California and to Wyoming. . . . . 8. *O. andina*

Plants with stems leafy from base; capsules terete, straight or coiled, 15-40 mm. long

Flowers small; petals 2.5-3.5 mm. long; calyx lobes 1.5-3.5 mm. long. . . . . 9. *O. contorta*

- Flowers larger; petals 5-15 mm. long; calyx lobes 3-12 mm. long. .... 10. *O. dentata*
- Capsules quadrangular; leaves 5-20 mm. wide, lanceolate to ovate
- Mature capsules oblong-pyramidal, 12 mm. long, nearly straight.—Guadalupe Island. .... 11. *O. guadalupensis*
- Mature capsules curved or contorted, 15-40 mm. long
- Flowers small; petals 1.5-7 mm. long. .... 12. *O. hirta*
- Flowers larger; petals 8-22 mm. long
- Plants of sea bluffs and inland; greenish (except for a desert form); cauline leaves lanceolate to lance-ovate, acute, wavy-margined, thin. .... 13. *O. bistorta*
- Plants of sea beaches, grayish to silvery (except in var. *nitida*); cauline leaves lance-oblong to orbicular-ovate, obtuse, not wavy-margined, thick. . 14. *O. cheiranthifolia*

#### DESCRIPTION OF SPECIES

1. *OENOTHERA REFRACTA* S. Wats., Proc. Am. Acad. 17:373. 1882.—Leveille, Monogr. Oenoth. 210. 1905; *Sphaerostigma refractum* (S. Wats.) Small, Bull. Torr. Bot. Club 23:192. 1896; Nels., BOT. GAZ. 40:58. 1905; *Oenothera deserti* Jones, Contr. West. Bot. 12:15. 1908; *Sphaerostigma deserti* (Jones) Heller, Muhlenbergia 9:68. 1913.

Annual, 15-40 cm. high, erect, with few to several divaricately spreading branches, usually glandular-puberulent and somewhat strigillose throughout; stems slender, commonly dark red, becoming whitish in age and with epidermis exfoliating; leaves largest near base of plant, but well distributed up to lowest flowers, oblanceolate to lanceolate to oblong-linear, entire or remotely and shallowly denticulate, green or slightly reddish, with callous tip, blades 20-50 mm. long and 3-8 mm. wide, lowest narrowed into petioles 10-20 mm. long, upper subsessile, uppermost reduced to linear bracts; inflorescence racemose, sometimes paniculate, 5-15 cm. long; calyx tube linear-funnelform, 4-6 mm. long, finely pubescent within; calyx lobes lance-oblong, 5-6 mm. long, often reddish; petals suborbicular, 4-7 mm. long and 3.5-6 mm. wide, whitish; stamens almost as long as petals, longer filaments 3.5-4.5 mm. long, shorter ones scarcely 1 mm. less; anthers 2-2.5 mm. long, pale yellow; style pubescent in

lower half, 10–13 mm. long, exceeding corolla; stigma about 1 mm. wide; capsule linear, commonly refracted or spreading, and straight or curved, sometimes coiled, 30–50 mm. long, about 0.5 mm. thick, generally not conspicuously beaked; seeds pale, linear, 1 mm. long.

Type locality (first specimen cited), "first collected by BIGELOW near the Colorado." Ranging on the deserts from Southern Utah to Arizona and Southern California. Representative material: UTAH, Bear Valley, *Palmer* 165 (G, NY); NEVADA, Hole-in-the-Rock, *Jones* 5036r (P); Rhyolite, *Jones* in 1907 (P); Bunkerville, *Jones* 5027 (P); Gold Mt., *Purpus* 5993 (C); Moapa, *Goodding* 2205 (C, G, W.); Las Vegas, *Goodding* 2239 (G, W); Overton, *Heller* in 1912 (S); ARIZONA, Chimehuevis, *Jones* in 1903 (P); Beaverdam, *Jones* 5024q (P); Ft. Mohave, *Cooper* in 1860–61 (G); Grand Canyon, *Gray* in 1885 (G); gravelly hills in the Colorado, *Bigelow* in 1854, type coll. (G, NY); Parker, *Wootton* in 1913 (US); CALIFORNIA, near Bishop, *K. Brandegee* in 1913 (C); Inyo, *T. S. Brandegee* in 1891 (C); Funeral Mts., *Jones* in 1907 (P); Cave Spring, *Jones* in 1924 (P); Kelso, *Jones* in 1906 (P); Daggett, *Parish* 9778 (C, S); Newberry, *Munz* et al. 4190 (P); Needles, *Jones* in 1884, type *O. deserti* (C, P); Chuckwalla Mts., *Munz & Keck* 4916 (P); Salton, *Hall* 5840 (C, S); Dos Palms Spring, *Munz* 9947 (P); San Felipe Creek, *Eastwood* 2753a (G).

2. *OENOTHERA CHAMAENERIOIDES* Gray, Pl. Wright. 2:58. 1853. —*O. chamaenerioides* Gray, in Wats. Proc. Am. Acad. 8:593. 1873; Leveille, Monogr. Onoth., 229. 1905, in part; *Sphaerostigma chamaenerioides* (Gray) Small, Bull. Torr. Bot. Club 23:189. 1896; *S. erythrum* Davidson, Bull. Soc. Calif. Acad. 1:118. pl. IX. 1902; *Oenothera erythra* (Davidson) Macbride, Contrib. Gray Herb 65:41. 1922.

Erect annual, 1–5 dm. high, usually branching near base; stems slender, often reddish, lower portions of stem and branches glandular-puberulent, somewhat viscid, upper portion more or less finely strigillose or glandular-puberulent; leaves rather thin, bright green, sometimes reddish, glabrate, blades of basal ones ovate-lanceolate to lanceolate, 4–8 cm. long, 1–2.5 cm. wide, entire, obtuse at apex, gradually attenuate at base with narrowly winged petioles 1–3.5 cm. long; lower cauline leaves somewhat reduced, upper ones below inflorescence becoming subsessile, those of inflorescence reduced to acute linear bracts, 10–20 mm. long; inflorescence a simple corymbose raceme, elongating in fruit and becoming as much as 20 cm. long; calyx tube narrowly funnelform, 2.5–3 mm. long, pubescent within; calyx lobes lance-ovate, 2.5 mm. long; petals white, often

drying pinkish, spatulate, ca. 3 mm. long; stamens subequal, glabrous; filaments almost 2 mm. long; anthers oblong, about 1 mm. long; style 3.5–4 mm. long, shorter than petals, pubescent on lower half; stigma 1 mm. wide; capsule terete, linear, divaricately spreading, scarcely if at all beaked, 25–50 mm. long, 0.5 mm. thick; seeds pale, linear, about 1 mm. long.

Type locality, near El Paso, Texas; ranging from there westward to California and Utah; growing only in deserts. Representative material: TEXAS, El Paso, *Jones* 3751 (C, P), *Wright* 1377, type (G); Frontera, *Bigelow* 373 (NY): SONORA, bed of Rio de Sonora, *Rose, Standley & Russell* 12413 (US): ARIZONA: Clifton, *Davidson* 244, type coll. *O. erythra* (C); Phoenix, *Jones* in 1903 (P); Tucson, *Thornber* 508 (C); Nogales, *T. S. Brandegee* in 1892 (C); Yucca, *Jones* 3934 (P, W); Wickenburg, *Jones* in 1903 (P); Beavertown, *Jones* 5024 r (P): UTAH, Dugway, *Jones* in 1891 (P); St. George, *Jones* 1623 (C, G, NY, P, W); southern Utah, *Parry* 71 (G, NY): NEVADA, Truckee Pass, *Kennedy* 1307 (W); Rhyolite, *Jones* in 1907 (P); Empire City, *Torrey* 100 (G); Calientes, *Jones* in 1904 (P): CALIFORNIA, Pleasant Canyon, Panamints, *Hall & Chandler* 6964 (C, P); Shepherd's Canyon, *Jones* in 1897 (P); Daggett, *K. Brandegee* in 1914 (C); Keyes Ranch, *Jones* in 1927 (P); Needles, *Munz & Harwood* 3632 (P); Goffs, *Jones* in 1903 (P); Chuckwalla Mts., *Munz & Keck* 4853 (C, P); Palm Canyon, *Spencer* 1465 (P); Mt. Springs Grade, *Peirson* 2898 (FP, P); San Felipe, *T. S. Brandegee* in 1895 (C); Yaqui Well, *Jones* in 1926 (P): LOWER CALIFORNIA, El Llano de Santana, *T. S. Brandegee* in 1889 (C).

The reduction of *O. erythra* needs no comment; there are no characters separating *O. erythra* from *O. chamaenerioides*.

3. OENOTHERA MINOR (Nelson), n. comb.—*Sphaerostigma minus* (given as *minor*) Nelson, Bull. Torr. Bot. Club 26:130. 1899.

Annual, more or less canescent-strigillose throughout (though leaves may be glabrate), scarcely if at all glandular; stems simple and erect or usually with several subequal ascending stems, somewhat reddish, slender, 5–30 cm. high, only tardily exfoliating; basal leaves largest, blades spatulate to oblanceolate to elliptic-ovate, subentire, acute to obtuse, 3–15 mm. wide, 5–25 mm. long, narrowed gradually into slender petioles 5–20 mm. long; upper leaves narrower and smaller, uppermost becoming linear-lanceolate foliose bracts; flowers borne singly in axils of almost all leaves, upper ones grouped in spicate inflorescence; calyx tube funnelform, 1–2 mm. long, pubescent within; calyx lobes narrowly ovate, 1–2 mm. long; petals "yellowish" to ochroleucous, often drying reddish, narrowly obovate,

1-2 mm. long; stamens strongly biseriate, the longer filaments twice the shorter ones and ca. two-thirds length of petals; anthers rounded, glabrous, 0.5-1.0 mm. long; style glabrous, almost equaling petals, 1.5-3 mm. long; stigma 0.5-1 mm. broad; capsule 10-25 mm. long, more or less contorted, gradually narrowed from base, often beaked; seeds narrowly obovoid, somewhat angled, grayish, ca. 1 mm. long.

#### KEY TO VARIETIES

Calyx tube 2 mm. long; calyx lobes and petals 2 mm.; capsules 18-25 mm. long; ranging from Wyoming to Nevada and Idaho. . . . 3a. *O. minor* var. *typica*  
 Calyx tube about 1 mm. long; calyx lobes and petals 1-1.2 mm.; capsules 10-18 mm. long; Eastern Washington and Oregon. . . . 3b. *O. minor* var. *cusickii*

3A. *OENOTHERA MINOR* (Nelson) Munz var. *typica*, n. nom.—  
*Sphaerostigma minus* (given as *minor*) Nelson, l. c.; *S. nelsonii* Heller, *Muhlenbergia* 1:1. 1900; Nelson, *BOT. GAZ.* 40:57. 1905; *Oenothera alyssoides* var. *minutiflora* S. Wats., *Bot. King Rep.*, 111. 1871; *Proc. Am. Acad.* 8:591. 1873; *Sphaerostigma alyssoides* var. *minutiflorum* (S. Wats.) Small, *Bull. Torr. Bot. Club* 23:192. 1896; *Sphaerostigma minutiflorum* (S. Wats.) Rydb., *Bull. Torr. Bot. Club* 33:146. 1906; *Oenothera chamaenerioides* var. *torta* Leveille, *Monogr. Onoth.*, 230. 1905, in part; *Sphaerostigma tortum* (Leveille) Nelson, *BOT. GAZ.* 40:60. 1905; *S. tortum* var. *eastwoodae* Nelson, *BOT. GAZ.* 40:61. 1905; not *Oenothera minutiflora* D. Dietr., *Syn. Pl.* 2:1285. 1840; not *Sphaerostigma minutiflorum* F. & M., *Ind. Sem. Hort. Petrop.* 2:50. 1835.

Calyx tube 2 mm. long; calyx lobes and petals 2 mm. long; style 3 mm., capsules 18-25 mm. long.

Type locality, Green River, Wyoming.—WYOMING, Ft. Steele, *Nelson* 3138 (W); Rock Springs, *Nelson* 9085 (W); Granger, *Nelson* 4691, type coll. *O. torta* (C, G, W); Green River, *Nelson* 4707 (P, W), 3047, type coll. *O. minor* (G, W), *Jones* in 1896 (P), *Payson & Armstrong* 3211 (P, W); Solon, *Williams* in 1897 (G); COLORADO, Grand Junction, *Eastwood* in 1892, type coll. var. *eastwoodae* (C, G, S); UTAH, Salina Canyon, *Jones* 5422i (P); Chepeta Well, *Jones* in 1908 (P); Milford, *Goodding* in 1902 (W), *Jones* in 1880 (P); Manti, *Jones* 5518 (C, P, W); Vermillion, *Jones* in 1901 (P); Hernansville, *Jones* in 1891 (P); Dutch Mt., *Jones* in 1891 (P), in 1900 (P); Black Rock, *Jones* 1958 (P), *Watson* 421 (G); Granite Mts., Tooele Co., *Jones* in 1900 (P); NEVADA, Shoshone, *Jones* in 1881 (P); Battle Mt., *Jones* in 1882 (P); Furber, *Jones* in 1891 (P); Oreana,



Jones in 1903 (P): IDAHO, Salmon, E. B. & L. B. Payson 1786 (G, W); Challis, Macbride & Payson 3233 (G, W).

In taking up the name *O. minor* for Watson's variety *minutiflora*, I am using the oldest species name for the concept, and am combining NELSON's and LEVEILLE's *torta* with *minor* (*Nelsonii* of HELLER). NELSON's specimens of *minor* are somewhat immature, and do not have the characteristic spreading branches of older plants. A distinction is made also in flower color, being given as yellow for *minor* and white for *minutiflora*. Careful study has failed to reveal any morphological characters, and dried plants do not show any color characters. I am convinced that the small-flowered plants of Wyoming and adjacent regions form one concept which is amply distinct from *alysoides* for specific rank, but not divisible into *minor* and *torta* and *eastwoodae*.

3B. *OENOTHERA MINOR* var. *cusickii*, n. var.—Smaller-flowered than var. *typica*, having calyx tube 1 mm. long; calyx lobes and petals 1–1.2 mm.; style 1.5 mm.; capsules 10–18 mm. long. (Tubus calycis 1 mm. longus; lobis calycis et petalis 1–1.2 mm. longis; capsulis 10–18 mm. longis.)

Type, Malheur River and adjacent hills, June 6, 1901, W. C. Cusick 2545 (University of California Herbarium 35348; cotypes at Gray, Pomona, and Wyoming): WASHINGTON, Moxee to N. Yakima, Griffiths & Cotton 41 (US); Coulee City, Piper 3889 (P, US, WS); Junction Crab & Wilson Creeks, Sandberg & Leiberg 262 (C, G, WS); Wilson Creek, Sandberg & Leiberg in 1893 (C); Morgans Ferry, Yakima River, Suksdorf 310 (G): OREGON, Malheur, Cusick 1223 (G), cited as 1228 by Leveille, Monogr. Onoth., 230, 1905 in his var. *torta*; Vale, Mathew Co., Leiberg 2051 (G); north end of Sumner Lake, Eggleston 6857 (US): IDAHO, Ft. Hall, Coulter in 1872 (US); a collection at Devil Creek, Owyhee Co., Idaho, by Nelson & Macbride 1747 (G, W) is intermediate between var. *cusickii* and var. *typica*. The variety *cusickii* has been included in *alysoides* var. *minutiflora* and in *torta*.

4. *OENOTHERA NEVADENSIS* Kellogg, Proc. Calif. Acad. 2:224. fig. 70. 1863.—*Sphaerostigma nevadense* (Kell.) Heller, Muhlenbergia 6:51, with cover illustr., 1910; *Sphaerostigma tortuosum* Nelson, Proc. Biol. Soc. Wash. 17:95. 1904; BOT. GAZ. 40:61. 1905; *Oenothera gauraeflora* var. *vermiculata* Jones, Contr. West. Bot. 12:16. 1908; *O. gauraeflora* var. *caput-medusae* Leveille, Monogr. Onoth., 226. 1905.

Annual, glabrate to finely and sparingly strigillose, especially on capsules, forming a small simple erect tuft, 2–5 cm. high, or in larger plants with several additional naked prostrate branches from 3–10 cm. long and each bearing a tuft or spike of leaves and flowers at the

tip; leaves narrowly oblanceolate, 10–35 mm. long, 3–7 mm. wide, glabrous, green, entire, not strongly veined, rounded or obtuse at apex, gradually narrowed at base to slender petioles 1–3 cm. long, or upper ones subsessile; uppermost reduced to leafy bracts; inflorescence spicate, usually crowded, sometimes elongate; calyx tube lance-ovate, sparingly pubescent, ca. 3 mm. long; petals white, orbicular-ovate, 3.5–5 mm. long; stamens in 2 series, the longer alternating with and equaling the petals, the shorter two-thirds as long; anthers oblong, 1.5 mm. long; style about 6 mm. long, equal to or slightly exceeding petals, glabrous; stigma 0.5 mm. broad; capsules 10–12 mm. long, 1.5–2 mm. thick, quadrangular with ridge running along middle of each of the four faces, swollen at base, gradually narrowed toward slender beak, coiled and twisted, usually in dense masses; seeds pale gray, ca. 1 mm. long, linear-obovoid.

Type locality not given. Ranging through Washoe and Ormsby counties in Nevada and possibly in adjacent California: NEVADA, hills north of Carson, *Stretch* in 1865 (NY); Wadsworth, *Kennedy* 2053 (S); Empire City, *Jones* 3862 (C, P, US, W), no. 4 (G); between Truckee Valley and Spanish Springs Valley, *Brandegee & Kennedy* in 1913 (C); Truckee Pass, *Kennedy* 766, type coll. *S. tortuosum* (C, S, W), 1592 (C), *Heller* 8647 (G, S); Reno, *Hillman* in 1894 (C), *Jones* in 1897, type coll. var. *vermiculata* (C, P, US), *Jones* in 1903 (P), *Cowgill* in 1901 (W), *Brandegee* (S), *Heller* 9697 (G, S); CALIFORNIA (?), *Lemmon* 102 (NY): fortunately the habit is so striking that good figures have been given with two of the names given to this plant, and specimens have been available for the others, so there is no question as to synonymy.

5. *OENOTHERA ALYSSOIDES* H. & A., Bot. Beechey Voy. 340. 1838.—Annual, usually branching from base, central stem erect, others ascending, and curved at tip, closely and finely pubescent throughout or short-villous especially at base, often glandular; stems rather slender, 5–35 cm. high, simple or again branched; lowest leaves oblanceolate to ovate-lanceolate, 15–40 mm. long, 5–15 mm. wide, entire or remotely denticulate, acute to obtuse, gradually narrowed into slender petioles of about same length; cauline leaves gradually reduced, subsessile; those of inflorescence still smaller, gradually becoming more bractlike; inflorescence racemose-spicate; calyx tube funnelform or sublinear, 3–8 mm. long; calyx lobes lance-ovate, 4–5 mm. long; petals white, 4–5 mm. long, orbicular-ovate; stamens glabrous, of two lengths, the longer ones somewhat shorter than the petals, with filaments 3.5 mm. long and anthers oblong and

1-1.5 mm. long; style 7-12 mm. long, equaling or slightly exceeding petals; stigma 0.5 mm. broad; capsule 14-23 mm. long, thickened at base (1.5 mm.), gradually attenuate toward beaklike tip, much coiled or only curved; seeds pale, linear-obovoid, minutely cellular-pitted.

### KEY TO VARIETIES

- Plant bright green in appearance, with only minute pubescence; calyx tube 2-3 mm. long, glabrous within; style glabrous; range in eastern Oregon and adjacent Idaho. . . . . 5a. *O. alyssoides* var. *typica*  
 Plant grayish in appearance, with conspicuous canescent or villous hairs; calyx tube 4-7 mm. long, pubescent within; style pubescent at base; from Utah to Nevada and Eastern California . . . . . 5b. *O. alyssoides* var. *villosa*

5A. *OENOTHERA ALYSSOIDES* H. and A. var. *typica*, n. nom.—  
*O. alyssoides* H. & A., l. c.; Hooker, *Icones Pl.*, t. 339. 1841; Watson, *Proc. Am. Acad.* 8:591. 1873, in part; *Sphaerostigma alyssoides* (H. & A.) Walp., *Rep.* 2:78. 1843; Small, *Bull. Torr. Bot. Club* 23:1896, in part; Nelson, *BOT. GAZ.* 40:62. 1905, in part; *Oenothera gauraeflora* of Leveille, *Monogr. Onoth.*, 223. 1905, in part; *Sphaerostigma implexum* Nelson, *BOT. GAZ.* 52:267. 1911.

General aspect of plant a bright green; pubescence of stems and leaves minute; flowers white, often drying yellowish; calyx tube 2-3 mm. long, glabrous within; style glabrous; capsules much coiled and twisted.

Type locality, "Pine Creek, Snake Country;" ranging in Eastern Oregon and adjacent Idaho: OREGON, desert region, E. Oregon, *Cusick* 1940 (G, WS); Malheur River, 40 miles above Ontario, *Griffiths & Morris* 899 (US); Mathew Valley, *Leiberg* 2257 (C, P, G); Ontario, *Leiberg* 2006 (C, G); base of Steins Mt., *Howell* in 1885 (C, G, WS); Old Ft. Smith, *Griffiths & Morris* 402 (US): IDAHO, Emmett, *Macbride* 890 (C, G, P, S, W); Falk's Store, Canyon Co., *Macbride* 27, type coll.; *S. implexum* (C, G, W, WS); King Hill, *Nelson & Macbride* 1098 (C, G, P, S, W); Glens Ferry, *Jones* in 1911 (P); Weiser, *Jones* in 1899 (P): "CALIFORNIA," *Douglas* (G), probably type material, mislabeled as to locality and really from Snake Country.

As here restricted, *O. alyssoides* var. *typica* is much less inclusive than *O. alyssoides* usually is. Instead of using simply a villous condition to distinguish the var. *villosa*, I am using length of hypanthium, pubescence within the hypanthium, and pubescence on base of style, with a canescent pubescence on stems and leaves whether it be long or short. I have seen several intergrades, having styles weakly pubescent and hypanthium intermediate in length:

Nampa, Idaho, *Mulford* in 1892 (G); Humboldt Mts., Nevada, *Torrey* 99 (G); Humboldt River, *Beckwith* 33 (G); Summit Lake, N. Nevada, *Griffiths & Morris* in 1901 (US).

5B. *OENOTHERA ALYSSOIDES* var. *VILLOSA* S. Wats., Proc. Am. Acad. 8:591. 1873.—*Sphaerostigma alyssoides* var. *macrophyllum* Small, Bull. Torr. Bot. Club 23:192. 1896; Nelson, BOT. GAZ. 40:62. 1905; *S. macrophyllum* (Small) Rydb., Bull. Torr. Bot. Club 40:66. 1913; *S. utahense* Small, Bull. Torr. Bot. Club 23:191. 1896; Nelson, BOT. GAZ. 40:61. 1905; *Oenothera utahensis* (Small) Garrett, Spring Flora Wasatch Reg., 64. 1911; *O. gauraeflora* var. *hitchcockii* Leveille, Monogr. Onoth., 226. 1905; *Sphaerostigma hitchcockii* (Levl.) Nelson, BOT. GAZ. 40:226. 1905.

With grayish aspect because of canescent or villous hair; flowers white, usually drying or aging pink; calyx tube usually 4–8 mm. long, pubescent within; style pubescent on lower half; capsules often merely curved.

Type locality, near Salt Lake, *Stansbury*; ranging to Nevada and the Mohave Desert of California: UTAH: Salt Lake Co., *Garrett* 1156 (G, W); Milford, *Jones* 1773 (P), *Goodding* in 1902 (W); north of Salt Lake City, *Rydberg* 6164 (W); near Salt Lake, *Stansbury*, type coll. (G, NY); Promontory Point, N. Utah, *Watson* 420 (G); Fish Springs, *Jones* in 1892 (P); Nephi, *Goodding* 1107 (G, W); Dugway, *Jones* in 1891 (P); Marysville, *Jones* 5388f (P, US); S. Utah, *Parry* 68 (G); "Simpson's Park," July 6, 1859, type of *S. hitchcockii* (M): NEVADA, Trinity Mts. *Watson* 420 (G); Panaca, *Jones* in 1912 (P); Palisade, *Jones* in 1882 (P), *Stokes* in 1903 (US); Muncy, *Jones* in 1891 (P); Goldfield, *Tidestrom* 9764 (G); Currant, *Bentley* in 1916 (P, S, W); Good Springs, *Jones* in 1905 (P); Esmeralda Co., *Shockley* 324 (C, G); Pyramid Lake, *Kennedy* 2044 in part (C, S), *Tidestrom* 10668 (G): ARIZONA, Ft. Mojave, *Cooper* in 1860–1861 (G): CALIFORNIA, Amedee, *Jones* in 1897 (C); Lassen Co., *Eggleston* 7176 (US); Deep Springs Valley, *Ferris* 1357 (S); Silver Canyon, E. of Laws, *K. Brandegee* in 1913 (C, W); White Mts. E. of Laws, *Heller* 8238 (G, S, US); Benton, *Shockley* 113 (C).

As here used this variety is a rather comprehensive and variable concept. I am unable to divide it on the basis of leaf size or length of hair or on other characters that have been used. Typically it has the habit of *O. alyssoides* var. *typica*, that is, several spreading stems from the base and second racemose spikes with lowest leaves oblanceolate and cauline ones lanceolate. Variants are as follows: (1) with stems single and branched above and usually slender and smooth as if from shade, the *Jones* collection at Fish Springs, Utah and at Good Springs, Nevada; (2) with cauline leaves becoming ovate and suggesting *O. boothii*, *Jones* 5388f, *Rydberg* 6164, *Jones* at Panaca and at Muncy, Nev.; (3)

with capsules less coiled and suggesting *O. decorticans*, part of the Brandegee collection from east of Laws; (4) with unusually long and villous hair, *Beniley* at Currant, Nev., *Goodding* and *Jones* collections at Milford, Utah.

6. *OENOTHERA DECORTICANS* (H. & A.) Greene, Fl. Francisc., 217. 1891.—Annual, erect, simple or branching below, and with branches ascending or spreading, glabrous or glabrate below, finely pubescent and often glandular in the inflorescence; stems with shining whitish or reddish epidermis which exfoliates readily; leaves largely near base, bright green or tinged red, glabrous to finely pubescent, lance-ovate to oblanceolate, entire or shallowly denticulate, acute, 2–8 cm. long, 0.5–3 cm. wide, narrowed into petioles of about same length; cauline leaves gradually reduced up the stem, becoming more narrow and subsessile; those of inflorescence reduced to lanceolate foliose or minute bracts; spikes usually fairly compact, in fruit 5–30 cm. long; calyx tube 4–6 mm. long, narrow, pubescent within; calyx lobes lance-ovate, 3.5–5 mm. long; petals white or reddish, obovate to suborbicular, 3.5–5.5 mm. long; filaments glabrous, not conspicuously unequal, almost as long as petals, anthers round-oblong to linear-oblong, 1–2 mm. long, glabrous; style almost equal to petals, pubescent on lower half; stigma about 0.5 mm. broad; capsule subfusiform, thickest in lower half, attenuate into slender beak, 1.5–3 mm. thick near base, 15–25 mm. long; seeds ash-colored, linear-obovoid, somewhat angled, minutely pitted, 1 mm. long.

#### KEY TO VARIETIES

Petals 3.5–4 mm. long, red, obovate, distinctly longer than wide; capsules curved with spreading tip; Mts. about western edge of Mohave Desert above 4000 ft. alt. .... 6a. *O. decorticans* var. *rutila*

Petals over 4 mm. long, usually whitish, but liable to turn red in age

Capsules not over 2 mm. thick at base, not conspicuously quadrangular nor thickened and indurated at angles, scarcely woody; petals usually distinctly longer than wide; plants tall, 20–50 cm., and fairly slender

Fruits with simple curve about one-third from base, so that tips spread away from stem; base of capsule ca. 2 mm. thick; exfoliating epidermis of stem straw- or flesh-colored; Monterey and Stanislaus Counties, California, south to Los Angeles and Bakersfield. .... 6b. *O. decorticans* var. *typica*

Fruits often more contorted so that tips point down; base of capsules 1–1.5

mm. thick; epidermis of stem white; Mohave Desert east to Nye County, Nevada, and occasional on Colorado Desert

6c. *O. decorticans* var. *desertorum*

Capsules 3 mm. thick at base, conspicuously quadrangular and much thickened and indurated at angles and with additional less prominent shallow ridge on each face between the angles, quite woody; plants low and coarse, rarely over 15–20 cm. high; exfoliating epidermis white; petals suborbicular, about as wide as long; deserts from Victorville and Barstow, California, to St. George, Utah, and to Mexican boundary

6d. *O. decorticans* var. *condensata*

6A. *OENOTHERA DECORTICANS* var. *rutila* (Davidson), n. comb.—

*O. rutila* Davidson, *Erythea* 2:62. 1894; *Sphaerostigma rutilum* (Davidson) Parish, *Erythea* 6:89. 1898; Nelson, *BOT. GAZ.* 40:63. 1905.

Rather slender-stemmed, considerably diffused with red; flowers small; petals 3.5–4 mm. long, red, distinctly longer than wide; capsules as in var. *typica*.

Type locality, Big Rock Creek, San Gabriel Mts., Los Angeles County, California; an ill-defined variety ranging along desert side of San Gabriel Mts., and approached by material from Mt. Pinos and the Panamints, California; Big Rock Creek, *Davidson* in 1893, type collection (G, S), *Davidson* 1605, in 1906 (C, S), *Munz* 6829 (P); Lytle Creek, *Hall* 1474 (C, S): the following collections approach this variety: Mt. Pinos, *Hall* 6477 (C) with small flowers but not so red; Seymour Creek, Mt. Pinos, *Peirson* 3218 (P), *Munz* 6998 (P) with flowers somewhat larger and not so red; Griffins, *Elmer* 3734 (G) with small but less red flowers; Panamint Canyon, *Jones* in 1897 (P) with red but larger flowers; and Shepherds Canyon, *Jones* in 1897 (P) with larger and less red ones; I have not seen the *Coville* and *Funston* collections from the Panamints (Contr. U.S. Nat. Herb. 4:105. 1893) listed under *O. gauraeiflora* by COVILLE.

6B. *OENOTHERA DECORTICANS* (H. & A.) Greene var. *typica*, n. nom.—*Gaura decorticans* H. & A., *Bot. Beechey*, 343. 1838; *Oenothera decorticans* (H. & A.) Greene, *Fl. Francisc.*, 217. 1891; *Sphaerostigma decorticans* (H. & A.) Small, *Bull. Torr. Club* 23:191. 1896; Nelson, *BOT. GAZ.* 40:60. 1905. *Oenothera alyssoides* var. *decorticans* (H. & A.) Jepson, *Man. Calif.*, 686. 1925, in part; *Oenothera gauraeiflora* T. & G., *Fl. N. Am.* 1:510. 1840; Watson, *Proc. Am. Acad.* 8:591. 1873; Leveille, *Monogr. Onoth.*, 223. 1905, in part.

Rather large plants, commonly 30–50 cm. tall; exfoliating epidermis of stems not a dead white, but somewhat straw- or flesh-

colored; petals about 5 mm. long, distinctly longer than wide; capsules about 2 mm. thick near base, somewhat rounded in cross-section and with tips bending away from stems.

Type locality, Monterey; ranging along the coast from Monterey to Los Angeles counties and in the San Joaquin Valley: CALIFORNIA, without locality, *Douglas*, type material of *O. gauraeflora* and probably of *O. decorticans* (G); Corral Hollow, Alameda Co., *Brewer* 1218 (C, G); San Benito, *Dudley* in 1899 (S); Porcines, *Abrams & Borthwick* 7997 (S); Hernandez, *Lathrop* in 1903 (S); Paloma Creek, Monterey Co., *Ferris* 1945 (S); Soledad, *Ferris* 4143 (P, S); Mission Valley, *Hall* 10013 (C, G, W); San Antonio Canyon, *Brewer* 579 (C, G); Parkfield, *Munz* 9185 (P); Paso Robles, San Luis Obispo Co., *Barber* in 1899 (C, P, W), *Jones* in 1909 (P), *K. Brandegee* in 1911 (C), *Munz* 10120 (P); Santa Barbara Co., *Baker* in 1895 (C); Bardsdale, Ventura Co., *Hall* 3111 (C, P, W); Sespe Creek, *Abrams & McGregor* 176 (G, S); Bouquet Canyon, L. A. Co., *Munz* 10004 (P); Cuddy Canyon, Mt. Pinos, *Dudley & Lamb* 4505 (S) Gormans Station, *Dudley & Lamb* 4438 (P, S); Caliente Creek, *Munz* 8995 (P); Bakersfield, *Davy* 1784 (C); La Puerta Creek, *Elmer* 4372 (S); Greenhorn Range, *Hall & Babcock* 5093 (C); Alcalde, *T. S. Brandegee* in 1891 (C).

6C. OENOTHERA DECORTICANS var. *desertorum*, n. var.—Habit more slender than in var. *typica*; epidermis whiter; flowers as in *typica*; capsules more slender, 1–1.5 mm. thick at base, more contorted so that the tips often curve downward. (Cutis caulium alba; capsulis tenuibus, 1–1.5 mm. crassis basilare, contortis et deflexis.)

Type, 10 miles southwest of Garlic Springs, Mohave Desert, California, April 8, 1924, *Munz & Keck* 7881 (Pomona College Herb. no. 48926); ranging over the western half of the Mohave Desert into Owens Valley, California, and to Rhyolite, Nevada; occasional on the Colorado Desert of California: NEVADA, Rhyolite, *Heller* 9682 (G, S): CALIFORNIA, Palm Springs (Agua Caliente), *S. B. & W. F. Parish* 628 (C), *Jaeger* in 1925 (P); Victorville, *Munz* 2586 (S), *Jones* in 1903 (P); Rabbit Springs, *Parish* 4955 (S); Box S Ranch, *Jones* in 1926 (P); Barstow, *K. Brandegee* in 1915 (C), *Johnston* 6459 (P); Hesperia, *Abrams* 2170 (P, S); Muroc, *Peirson* 7271 (P); Lancaster, *Elmer* 3985 (G, P, S); Willow Springs, *F. Grinnell* 424 (US); Mohave, *Dudley* 515 (S); Randsburg-Rand, *K. Brandegee* in 1913 (C); Owens Lake, *Purpus* 1932 (C); Argus Mts., *Purpus* 5410 (C, G); Keeler, *Coville & Funston* 859 (G, S); Bissell, *K. Brandegee* in 1912 (C); Lone Pine, *Jones* in 1927 (P). In the Owens Valley var. *desertorum* grades insensibly into *O. alyssoides* var. *villosa*; on the whole it can be distinguished by its less contorted fruits, narrower petals, less pubescence, and by its tendency to have a central stalk instead of several stems from the very base as does *alyssoides*. Of the collections cited, however, *Coville & Funston* 859 and *Purpus* 5410 approach *O. alyssoides* var. *villosa*.

6D. *OENOTHERA DECORTICANS* var. **condensata**, n. var.—Stems low and thick, usually not over 15–18 cm. tall and with pure white epidermis; petals 4–5 mm. long, suborbicular; capsules woody, much thickened, about 3 mm. at base, quadrangular and with supplementary ridge down the middle of each face. (Caules crassi et 10–18 cm. alti; petalis 4–5 mm. longis, suborbicularibus; capsulis ligneis, circa 3 mm. crassis, quadrangularibus.)

Type, Dos Palmos Spring, Colorado Desert, Riverside County, California, Jan. 31, 1926, *Munz* 9960 (Pomona College Herb. no. 98708); ranging through the eastern half of the Mohave Desert and through the Colorado Desert, eastward to St. George, Utah: UTAH, S. Utah, *Parry* 67 (G); St. George, *Jones* in 1880 (P); NEVADA, Moapa, *Goodding* 2189 (C, G, W), *Jones* in 1904 (P); Mormon Mts., *Kennedy & Goodding* 128 (C); Good Springs, *Jones* in 1905 (P); Las Vegas, *Goodding* 2326 (W); Tuly's Ranch near Las Vegas, *Goodding* 2346 (G, W); ARIZONA, Cactus Station, *W. F. Parish* 66 (S); Valley of the Colorado, *Palmer* 135 (C); Stern's Ferry, *Jones* 5032c (P); Beaverdam, *Jones* 5024s (P); CALIFORNIA, Funeral Mts., *Jones* in 1907 (P); Randsburg-Rand, *K. Brandegee* in 1913 (C); Kelso, *Jones* in 1906 (P); Daggett, *K. Brandegee* in 1914 (C); Bagdad, *Munz, Harwood, & Johnston* 4291 (P); Needles, *Jones* 3787 (C, P, W); Blythe Junction, *Munz & Harwood* 3591 (P); Shavers Well, *Munz & Keck* 4747 (P); Chuckwalla Bench, *Hall* 5888 (C); Coachella, *Hall* 5804 (C); Palm Springs, *Spencer* 1445 (P); Signal Mt., *Abrams* 3174 (G, S); Borregos Springs, *K. Brandegee* in 1899 (C); Coyote Wells, *Spencer* 244 (G, P); Calexico, *Davy* 7993 (C). In describing this variety I admit that flowering material is somewhat difficult to distinguish, although the low habit of growth and the broad petals are fairly good characters. After the plants have somewhat mature capsules, however, they are very distinct. The thick woody stems with heavy capsules are quite persistent, and their dried remains are left standing on the desert for some time after the plant is dead, reminding one of a pine cone from which the scales have been broken and the shreddy scale bases and central axis are left.

7. *OENOTHERA BOOTHII* Dougl., ex Hook., Fl. Bor. Am. 1:213. 1834.—Annual, glandular-pubescent to glandular-villous throughout, erect, 1–4 dm. high, usually with central stem more prominent than the branches, which are few to several and spreading or ascending; leaves ovate or oblong-ovate, fairly evenly distributed, shallowly toothed, acute to obtuse, lowermost narrowed into petioles 1–3 cm. long, the blades 2–5 cm. long and 1–2.5 cm. wide; upper leaves gradually reduced and becoming sessile; those of inflorescence more or less foliose bracts; inflorescence racemose-spicate, often quite congested, elongating in fruit and becoming 5–15 cm. long; calyx



tube narrowly funnelliform, 2-8 mm. long, pubescent within; calyx lobes lance-ovate, 2.5-7.5 mm. long; petals white, pinkish in age, clawed, obovate, 2.5-8.5 mm. long; stamens subequal; filaments glabrous, almost as long as petals; anthers rounded-oblong to linear, glabrous, 1-2 mm. long; style pubescent on lower half, 4-15 mm. long, equaling petals; capsules short, 10-15 (-18) mm. long, usually ascending in lower half and with terminal portion spreading but not contorted, much thicker at base (1.5-2 mm.) than in terminal portion; seeds brownish, rhomboid-prismatic, minutely cellular-pubescent, about 1 mm. long, immature seeds linear-obovoid, grayish.

#### KEY TO VARIETIES

Flowers small; petals 1.5-2.5 mm. long; Eastern Washington and Oregon.

7a. *O. boothii* var. *pygmaea*

Flowers larger; petals 3.5-9 mm. long; Idaho and S. E. Washington to Nevada and Eastern California . . . . . 7b. *O. boothii* var. *typica*

7A. OENOTHERA BOOTHII var. PYGMAEA (Dougl.) T. & G., Fl. N. Am. 1:510. 1840.—*O. pygmaea* Dougl., ex Lehmann in Hook., Fl. Bor. Am. 1:213. 1834; *Sphaerostigma boothii* var. *pygmaeum* (Dougl.) Walp., Rep. 2:78. 1843.

Flowers small; calyx tube 1.5-2.5 mm. long; calyx lobes 1.5-2.5 mm. long; petals 1.5-2.5 mm. long, narrowly obovate; style 4-5 mm. long.

Type locality, "near the branches of Lewis and Clark's River, lat. 46° north:" WASHINGTON, Rock Island, *Sandberg & Leiber* 441 (C, G, P, WS): OREGON, Pine Creek, *Leiber* 199 (C, G, US); Muddy Station, John Day Valley, *Howell* in 1885 (G, US, WS); Squaw Creek, *Henderson* 5411 (S). Photographs of the types of *O. boothii* and *O. pygmaea* Dougl. from the Kew Botanical Garden show that *O. boothii* has rather larger flowers, with petals about 3.5 mm. long, and the ovate merely serrate leaves of the more southern plants, which have largely gone under the name *Sphaerostigma senex*, and which commonly have petals ranging from 5 to 9 mm. long. The type of var. *pygmaea* has no flowers, but has the narrower and undulate-margined leaves of the smaller flowered plants of E. Washington and Oregon, and is quite like *Sandberg & Leiber* 441. After careful comparison of these photographs with herbarium specimens of the larger and smaller flowered series, I refer the name *O. boothii* var. *pygmaea* to the latter, and *O. boothii* var. *typica* to the former.

7B. OENOTHERA BOOTHII Dougl. var. **typica**, n. nom.—*O. boothii* Dougl., ex Lehmann in Hook., Fl. Bor. Am. 1:213. 1834; Watson,

Proc. Am. Acad. 8: 591. 1873, in part; *Sphaerostigma boothii* (Dougl.) Walp., Rep. 2: 77. 1843; Small, Bull. Torr. Bot. Club 23: 191. 1896, in part; Nelson, BOT. GAZ. 40: 61. 1905, in part; *Oenothera gaurae-flora* race *boothii* of Leveille, Monogr. Onoth., 226. 1905, in part; *Sphaerostigma senex* Nelson, Proc. Biol. Soc. Wash. 18: 173. June 29, 1905; *S. lemmoni* Nelson, BOT. GAZ. 40: 61. July 18, 1905.

Flowers larger; calyx tube 4–8 mm. long; calyx lobes 3–7 mm.; petals 3.5–9 mm. long, broadly obovate; style 5–14 mm. long.

Type locality, "on the high sandy and gravelly hills of Lewis and Clark's River;" ranging from Eastern Washington to E. California and W. Nevada: WASHINGTON, Snake River, Walla Walla region, *Brandegee* 785 (C, G); IDAHO, Shoshone Falls, *Henderson* in 1897 (W); Blue Lakes, *Palmer* 523 (US), 71 (US); those mentioned so far have flowers about the size of those in the type collection; the following are larger: Payette River, *Henderson* 3115 (US); OREGON, Snake River, *Nuttall* (G); NEVADA, without locality, *Wheeler* in 1872 (US); Virginia Mts., *Bailey* 419 (G, US); Lake Range, *Watson* 419 (G, US); Walker Lake, *Heller* 10912 (C, G, S, US); Truckee Range, *Bailey* in 1867 (G); Carson Lake, *Peirce* in 1926 (P, S); Ely, *A. E. Hitchcock* 1198 (US); Pyramid Lake, *True* 750, type of *S. senex* (W); *K. Brandegee & Kennedy* in 1913 (C), *Frandsen & Brown* in 1906 (C, W), *Hall* 10554 (C); Candelaria, *Shockley* 324 (C, S); Gold Mt., *Purpus* 5901 (C, P); Austin, *Hitchcock* 744 (US); UTAH: Diamond Valley, *Purpus* 6260 (C); ARIZONA, Cinder flats east of San Francisco Peaks, *Leiberg* 5808 (US); *Francisco Mt., Knowlton* 200 (US); CALIFORNIA: East flank of Sierra Nevada, *Lemmon* 103, type of *S. lemmoni* (M), in 1875 (US); Hesperia, *Abrams* 2166 (S); upper part of Mojave River, *S. B. & W. F. Parish* 1504 (G, S).

This variety is exceedingly variable as to pubescence, size, leaf shape, etc., and it is sometimes difficult to distinguish from *O. alyssoides* var. *villosa*, but on the whole its capsules are shorter and not coiled, and the foliage is more decidedly glandular. Mature seeds are quite characteristic. Plants from about Pyramid Lake, Nevada, have especially large flowers.

8. *OENOTHERA ANDINA* Nutt., ex T. & G., Fl. N. Am. 1: 512. 1840.—Low, erect, very slender-stemmed annuals, with very spreading branches from near the base or from above, finely canescent throughout, 2–15 cm. high and about as broad; lower stem and branches, in all except the smallest plants, rather free of leaves; leaves alternate, linear to oblanceolate-linear, entire, acute to obtusish at somewhat thickened apex, basally gradually attenuate into short indistinct petioles; blades 10–30 mm. long, 1–2.5 mm. wide, somewhat but not markedly reduced in upper part of plant, the upper ones rather crowded; flowers axillary in a rather crowded

corymb which becomes racemose in fruit; calyx tube 1-2 mm. long, sparsely pubescent; calyx lobes oblong-ovate, 1.5-2.5 mm. long; petals ovate to orbicular, sessile, 1.2-2.5 mm. long, yellow; stamens of 2 lengths, longer ones (including filaments) 1-2 mm. long, shorter ones about half as long; anthers 0.3-0.6 mm. long; style stout, 2.5-4.5 mm. long; stigma 0.4-0.7 mm. broad; capsules fusiform, somewhat quadrangular, 5-8 mm. long; seeds fusiform, smooth, brown, ca. 0.7 mm. long.

#### KEY TO VARIETIES

Flowers small; petals about 1.5 mm. long. . . . . 8a. *O. andina* var. *typica*  
 Flowers larger; petals about 2.5 mm. long. . . . . 8b. *O. andina* var. *hilegardii*

8A. OENOTHERA ANDINA Nutt. var. *typica*, n. nom.—*O. andina* Nutt., l. c.; Wats., Proc. Am. Acad. 8:594. 1873; Leveille, Monogr. Onoth., 181. 1905; *Sphaerostigma andinum* (Nutt.) Walp., Rep. 2:79. 1843; Small, Bull. Torr. Bot. Club 23:188. 1896; Nelson, Bot. GAZ. 40:56. 1905; *Oenothera andina* f. *tripetala* Leveille, l. c., 182.

Calyx tube about 1 mm. long; calyx segments 1.5 mm.; petals 1.5 mm. long, ovate; longer filaments 1. mm.; anthers 0.3 mm.; capsules 5-6 mm. long.

Type locality, "Dry plains in the Rocky Mountains, near the Black-Foot River." WYOMING: Cokeville, Nelson 4644 (G, W); Green River, Parry 111 (G, NY), Payson & Armstrong 3212 (P, W); UTAH, Heber Valley, Watson 425 (G, NY); Minersville, Stokes in 1903 (C); NEVADA, East Humboldt Mts., Watson 425 (G, NY); Havawat Mts., Watson 425 (NY); Empire City, Jones 3862 (C); Palisade, Jones 273 (P); IDAHO: Rocky Mts., Nuttall, type coll. (G, NY); Blackfoot, Jones in 1909 (C, P); Salmon, E. B. & L. B. Payson 1778 (G, W); Devil Creek, Owyhee Co., Nelson & Macbride 1746 (G, W); Squaw Creek, Boise Co., Macbride 806 (C, G, W); Tikura, Nelson & Macbride 1295 (C, G, P, W); Shoshone, Mulford in 1892 (NY); Weiser, Jones in 1899 (P); Pocatello, Henderson in 1897 (W); CANADA, Assiniboia, Pend d'Oreille Post, Macoun in 1895 (G); Medicine Hat, Macoun 7531 (G); WASHINGTON, Pasco, Piper 2994 (WS); Spangle, Piper in 1899 (WS); Omak, Jones in 1911 (C, P); Yakima, Brandegee in 1886 (G); Ellensburg, Piper 2675 (WS), Elmer 429 (P, WS); Wenatchee, Whited 86 (WS); Sprague, Sandberg & Leiberg 145 (C, G, WS); Klickitat Valley, Howell 1503 (C, NY, WS); OREGON, Steins Mts., Howell in 1885 (NY, WS); Baker City, Jones in 1902 (P); Chandlers Stage Station, Eggleston 6895 (US); CALIFORNIA, Goose Lake Valley, Austin in 1894 (C); Mt. Bidwell, Manning 384 (C); Shumway, Lassen Co., Austin & Bruce 2364 (C, S); Big Valley, Modoc Co., Baker & Nutting in 1894 (NY, C); Warners Mts., Peirson 6869 (P); Susanville, Jones in 1897 (P); Chat, Jones in 1897 (P).

8B. *OENOTHERA ANDINA* var. *hilgardii* (Greene), n. comb.—*O. hilgardi* Greene, Bull. Torr. Bot. Club 10:41. 1883; *Sphaerostigma hilgardi* (Greene) Small, Bull. Torr. Bot. Club 23:188. 1896; *S. andinum* var. *hilgardii* (Greene) Nelson, Bot. Gaz. 40:56. 1905.

Flowers larger; calyx tube 1.5 mm. long; calyx lobes 2 mm.; petals 2.5 mm. long, suborbicular; longer filaments 2.0 mm.; anthers 0.6 mm.; capsules 7–9 mm. long.

Type locality, "moist alkaline soil of the Klickitat Swale, Washington:" WASHINGTON, without locality, *Whited* 1093 (US); Yakima Region, *Brandegee* in 1882 (C); Coulee City, *Piper* 3907 (WS); Ellensburg, *Whited* 388 (US); Wilson Creek, *Sandberg & Leiberg* in 1893, in part (W); Wenatchee, *Whited* 1093 (WS); Wenas, *Griffiths & Cotton* 89 (WS); Cascade Mts., *Vasey* in 1889 (WS); Jct. Crab & Wilson Creeks, *Sandberg & Leiberg* 290 (C, G, WS); Omak Lake, St. John 7698 (WS); Riverside, St. John 7706 (WS); Walla Walla region, *Brandegee* 786 (G); E. Wash. Territory, *Hilgard* in 1882, type coll. (G): OREGON, Hayden Island, Columbia River, *Nelson* 2948 (G).

This variety is marked as an entity mostly by its larger floral parts, and is not any too distinct. PIPER (Contr. U.S. Nat. Herb. 11:405. 1906) recognizes var. *hilgardii* as a species, and distinguishes between it and *O. andina* on the basis of seeds as well as flower size, giving the seeds of the former as "pale, linear" and of the latter as "dark, clavate," a distinction I am unable to maintain. Furthermore, there is a certain amount of intergradation in flower size, as evidenced by the following collections which have petals about 2 mm. long: Wilson Creek, Wash., *Sandberg & Leiberg* in 1893, in part (C, P); Coulee City, *Piper* 3908 (WS); Juniper Springs, Mathew Co., Ore., *Leiberg* 2282 (C,G).

9. *OENOTHERA CONTORTA* Dougl. ex Hook., Fl. Bor. Am. 1:214. 1834.—*Oenothera torulosa* Leveille, Monogr. Onoth., 176. 1905, in large part.

Annual, slender-stemmed, 5–40 cm. high, rarely simple, usually with few to several subequal branches from near the base, these erect or ascending; stems usually reddish, glabrate to pubescent, leafy; leaves linear to lance-linear or linear-oblongate, subsessile, remotely denticulate, glabrate to pubescent or short-villous, acute, 5–25 mm. long, 1–3 mm. wide; lower leaves frequently with fascicles of smaller ones in axils or with short branches; uppermost leaves reduced to leafy bracts subtending the flowers; flowers few to many on a stem, but only one or two in anthesis at once; calyx tube obconic, 1–2 mm. long, glabrous or pubescent within and glabrate to glandular or strigillose without; calyx lobes lance-ovate, 1.5–3.5

mm. long, varying in pubescence with the calyx tube; petals bright yellow, aging red, narrowly obovate to obcordate, rounded, notched or apiculate, 2.5-3.5 mm. long, 1.5-3.5 mm. wide; longer stamens twice the shorter, glabrous, one-third to one-half length of petals; anthers rounded, 0.5-0.7 mm. long, erect; style equaling longer stamens, glabrous, 2-4.5 mm. long; stigma 0.6-1.0 mm. broad; capsules linear, cylindrical, often torulose, sessile or stalked, truncate or beaked, glandular or pubescent, straight or curved, 15-40 mm. long, 1 mm. or less thick; seeds brown, obovoid, 0.6-1.0 mm. long, minutely cellular-pitted.

The plants that I am including under this species offer an exceedingly puzzling and difficult taxonomy; they are found over practically all western North America, from British Columbia to Lower California and from the Rocky Mountains to the Pacific Ocean. What is apparently the same species occurs in the southern part of South America, but so far as I can ascertain, the name *contorta* has priority over all others. For the most part the species seems to be one that grows in sandy situations along the coast, rivers, sand banks, and the like. In North America I am proposing the recognition of five varieties, although by using very minute and inconstant characters, the number could be doubled.

### KEY TO VARIETIES

Plants low, commonly less than 15 cm. tall, with very slender glabrate or finely pubescent leaves less than 2 mm. wide

- Capsules sessile, 25-35 mm. long and ending in well-defined beak; E. Washington to W. Nevada and central E. California....9a. *O. contorta* var. *typica*
- Capsules distinctly pediceled, not beaked, 17-25 mm. long; E. Washington to Wyoming and the Mohave Desert of California

9b. *O. contorta* var. *flexuosa*

Plants taller or heavier, glabrous or pubescent

Stems densely pubescent with short appressed or incurved hairs; capsules 15-25 mm. long, not beaked; coastal Central California from Sonoma to Monterey counties.....9c. *O. contorta* var. *strigulosa*

Stems glabrous or with spreading pubescence; capsules usually over 25 mm. long

Plants spreading, coarse, grayish, with abundant spreading hair; leaves over 2 mm. wide; capsules sessile or nearly so, not beaked; W. Nevada and adjacent California.....9d. *O. contorta* var. *pubens*

Plants tall, erect, glabrous or some with spreading hair; capsule commonly beaked; leaves usually 1-2 mm. wide; from S. W. Oregon to Lower California, largely in interior valleys of the coastal drainage

9e. *O. contorta* var. *epilobioides*

9A. *OENOTHERA CONTORTA* Dougl. var. **typica**, n. nom.—*O. contorta* Dougl. ex Hook., l. c.; T. & G., Fl. N. Am. 1:510. 1840; *Sphaerostigma contortum* Walp. Rep. 2:78. 1843; Small, Bull. Torr. Bot. Club 23:189. 1896, in part; Nelson, Bot. Gaz. 40:58. 1905, in part.

Stems slender, glabrate to finely pubescent, usually 5–10, occasionally 25 cm. tall, spreading; leaves narrow, less than 2 mm. wide; capsules sessile, curved or straight, 25–35 mm. long, ending in a definite beak.

Type locality, "on sandy barren soils of the interior parts of the Columbia River," *D. Douglas*, from photograph of type at Kew (P); ranging east of the Cascade and Sierra Nevada Mountains from Washington to Western Nevada and Eastern Central California: CANADA, Cedar Hill, British Columbia, *Pineo* in 1896 (C); WASHINGTON, Rockland, *Suksdorf* 5098 (S, WS), 5099 (S, WS), 5101 (S, WS), 5100 (S, WS), Pasco, *Piper* 2965b (WS); Bingen, *Suksdorf* 2311 (C, G); Illia, *Lake & Hull* in 1892 (WS); OREGON, Hay Creek, *Leiberg* 204 (C, G), in 1894 (WS); the Dalles, *Sheldon* 10277 (G, P, S, WS), 10263 (G, P, S, WS), *Heller* 10091 (G, S), 9262 (S), *Sackley* (G), *Lyll* in 1860 (G); Umatilla, *Jones* in 1902 (P); IDAHO, Burnt River Canyon, *Cleburne* in 1883 (P); Big Willow, Canyon Co., *Macbride* 158 (G, W, WS), 158a (G, W); Weiser, *Jones* in 1899 (P), in 1900 (P); Boise, *Clark* 38 (C, G, P, S, W); New Plymouth, *Nelson & Macbride* 777 (W); NEVADA, Tonopah, *Shockley* 147 (P, S); Carson City, *Jones* in 1897 (P), *Anderson* in 1864 (US); Steamboat Springs, *Sonne* in 1887 (C); Empire City, *Jones* in 1882 (P); CALIFORNIA: Chat, *Jones* in 1897; Big Valley, Modoc Co., *Baker & Nutting* in 1894 (P).

This variety is much like var. *pubens*, but smaller and more delicate. It has at least four forms: (1) glandular in the inflorescence, *Sonne* at Steamboat Springs, *Heller* 10091, *Sheldon* 10263, and *Jones* at Umatilla and Carson City; (2) glabrous, *Suksdorf* 5098; (3) not glandular, with spreading pubescence, *Suksdorf* 5101, 5099, 5100, *Sheldon* 10277; and (4) not glandular and with appressed pubescence, *Suksdorf* 2311, *Clark* 38, *Macbride* 158. The low habit, however, and the slender, beaked, sessile capsules are quite constant.

9B. *OENOTHERA CONTORTA* var. **flexuosa** (Nelson), n. comb.—*Sphaerostigma contortum* var. *flexuosum* Nelson, Bot. Gaz. 40:58. 1905; *S. flexuosum* (Nelson) Rydb., Fl. Rocky Mts., 601. 1907; *Oenothera parvula* Nutt. in T. & G., Fl. N. Am. 1:511. 1840; *Sphaerostigma parvulum* Walp., Rep. 2:78. 1843; *S. filiforme* Nelson, Bot. Gaz. 40:57. 1905.

Stems and leaves as in var. *typica*; capsules slender, not over

0.5 mm. thick, distinctly pediceled, not attenuate into a beak, 17-25 (-30) mm. long, frequently curved into a half circle.

Type locality, Point of Rocks, Wyoming; ranging with the last but east to Wyoming and south to the Mohave Desert of California: WYOMING: Granger, *Nelson* 4698 (W); Pacific Creek, *Merrill & Wilcox* 602 (G, US, W); Point of Rocks, *Nelson* 4760, type coll. (G, W); Ft. Steele, *Tweedy* 4432 (US): UTAH, Deep Creek, *Jones* in 1891 (C, G, P), New River, in 1859, type of *S. filiforme* (M); Gold Hill, *Jones* in 1891 (P, W); Silver Reef, *Jones* 5149n (P); Sevier Bridge, *Jones* in 1880 (P); Thompsons Spring, *Jones* in 1891 (P); Detroit, *Jones* in 1891 (P): NEVADA, Steamboat Springs, *Stokes* in 1903 (C); Truckee Pass, *Kennedy* 1306 (C, NY, S, W); Cobre, *Jones* in 1906 (P), in 1907 (P); Candelaria, *Shockley* 247 (S): CALIFORNIA, Cactus Flat, San Bernardino Mts., *Jones* in 1926 (P), *Munz* 10519 (P); Kramer, *K. Brandegee* in 1913 (C); Argus Mts., *Hall & Chandler* 7089 (C); Bishop, *Heller* 8288 (C, G, S), *Jones* in 1927 (P), *K. Brandegee* in 1913 (C); Bishop Creek, *Hall & Chandler* 7244 (C, P); Coso Mts., *Coville & Funston* 938 (S); Lone Pine, *Jones* in 1927 (P), in 1897 (P, US); Lone Pine Creek, *Hall & Chandler* 7208 (C); Chat, *Davy* in 1897 (C); Amedee, *Jones* in 1897 (P): OREGON, Summer Lake, *Eggleston* 6861 (US); Blue Mts., *Cusick* in 1897 (G); Mathew Valley, *Leiberg* 2221 (C, G, P): IDAHO (?), Rocky Mts., "toward Lewis' River," *Nuttall*, type coll. of *O. parvula* (NY): WASHINGTON, Cascade Mts., *Vasey* in 1889 (G, WS); Jct. Crab & Wilson Creeks, *Sandberg & Leiberg* 263 (C, G, WS); Pasco, *Piper* 2965a (WS); N. Yakima, *Henderson* in 1892 (WS).

This variety is another variable group. The inflorescence may be (1) glandular, as in *Piper* 2965a, *Sandberg & Leiberg* 263, *Hall & Chandler* 7244, *Kennedy* 1306; or (2) strigillose, as in *Heller* 8288 and *K. Brandegee* near Bishop; or (3) glandular and strigillose, as in *Henderson* at North Yakima; or (4) glandular and with spreading pubescence, as in *Jones* at Cobre, Nevada. Then too, the capsules may be excessively long, as in the *Vasey* collection in the Cascades, which has them 4 cm. long. *Hall & Chandler* 7089, *Jones* from Lone Pine, and others suggest var. *pubens* by their pubescence; in fact many of the plants I refer here have formerly been called *pubens*, but it seems best to restrict that variety to plants of heavier stature and broader leaves.

9C. OENOTHERA CONTORTA var. PUBENS (S. Wats.) Coville, Contr. U.S. Nat. Herb. 4:104. 1893.—*O. strigulosa* var. *pubens* S. Wats., Proc. Am. Acad. 8:594. 1873; *Sphaerostigma contortum* var. *pubens* (S. Wats.) Small, Bull. Torr. Bot. Club 23:189. 1896, in part; *Nelson*, Bot. Gaz. 40:58. 1905; *S. pubens* (S. Wats.) Rydb., Bull. Torr. Bot. Club 33:146. 1906; *S. orthocarpum* *Nelson & Kennedy*, Proc. Biol. Soc. Wash. 19:155. 1906.

Coarse-stemmed plants with abundant spreading pubescence;

leaves rather broad, commonly over 2 mm. wide; capsules 1 mm. or more in diameter, 25–35 mm. long, sessile or subsessile, not beaked.

Type locality, Carson City, Nevada, if we select as the type *Watson* 423 (G). In his description, Watson cites first *Fremont* 219 from California in a list of several numbers; but on reference to Bot. King, 112. 1871, where Watson uses "*O. dentata* var." and which describes what he two years later called *pubens*, his own collection is seen to be nearest the true type. The *Fremont* collection probably belongs to var. *epilobioides*. As here restricted, var. *pubens* is found in western Nevada and adjacent California, as follows: NEVADA, Thomas Canyon, *Kennedy* 1872 (US); Empire City, *Jones* 3870 (W); Carson City, *Watson* 423 (G, US), *Anderson* 99 (G), 155 (G), in 1864 (US), *Baker* 971 (C, G, P, US, W); Currant *Bentley* in 1916 (S); Lake View, *K. Brandegee* in 1913 (C); Verdi, *Sonne* in 1888 (C); Reno, *Jones* in 1903 (P), *Diehl* in 1902 (P), *Hillman* in 1893 (P); Pyramid Lake, *Kennedy* 1015a, type of *S. orthocarpum* (W), 1211 (C, US); Hawthorne, *Jones* in 1882 (P); Virginia City, *Jones* in 1882 (P): CALIFORNIA, Sierra Nevada Mts., *Lemmon* in 1875 (US); Doyle Station, *Eggleston* 6741 (US), 6732 (US); Honey Lake, *Brandegee* in 1892 (C); Erskin Creek, *Purpus* 5365 (C, G).

9D. OENOTHERA CONTORTA var. *strigulosa* (F. & M.), n. comb.—*Sphaerostigma strigulosum* F. & M., Ind. Sem. Hort. Petrop. 2:50. 1835; *Oenothera strigulosa* T. & G., Fl. N. Am. 1:512. 1840, in part.

Stems densely pubescent, with short appressed or incurved hairs, and growing to be 15–30 cm. tall; capsules short, 15–25 mm. long, not beaked.

Type locality, "Nova California," ranging along the coast from Humboldt to Monterey counties: CALIFORNIA, *Horto Berol.*, in 1839 (G); *Horto Cantab.*, Anno 1843, e sem. Petrop. (G); Buckport, Humboldt Co., *Tracy* 2191 (C, G); Bodega Bay, *Heller & Brown* 5613 (G, P, S, W); Presidio, *M. S. Baker* in 1904 (P), *C. F. Baker* 711 (P); Golden Gate Park, *Davy* 185 (C); San Francisco, *Rattan* in 1878 (S), *Davy* in 1895 (C), *Greene* in 1893 (C); Lake Merced, *Dudley* in 1901 (S), *Davy* in 1896 (C), *Hall* 1788 (C), *Tracy* 1828 (P, W); Alameda, *Bioletti* in 1891 (C); Colma, *K. Brandegee* in 1905 (C); Antioch, *Rattan* in 1879 (S); Alhambra Valley, *Rattan* in 1887 (S); Holladay Hill, *Rattan* in 1887 (S); Watsonville, *Baker* 1951 (C, G, P, W); Bonnie Doon, Santa Cruz Co., *Dudley* in 1899 (S); San Lorenzo Road, *Dudley* in 1893 (S); Lone Mt., *Rattan* in 1877 (S); San Simeon, *Brandegee* in 1888 (C); Mt. Hamilton, *Elmer* 4625 (C, P, S, US); Bald Peak, *Dudley* 4200 (S); Pacific Grove, *Patterson & Wiltz* in 1907 (S); Carmel Valley, *Randall* in 1910 (S); Monterey, *Dudley* in 1894 (S), in 1901 (S); Del Monte, *Mason* in 1921 (S); Tassajara Road, *Abrams* 5646 (S); San Juan, *Brewer* 715 (C); Pismo Beach, *Abrams* 6513 (S); Santa Rosa Island, *Brandegee* in 1888 (C).



This is a fairly distinct variety with its appressed pubescence and coastal range, but does intergrade to some extent with var. *epilobioides*; *Elmer* 4625 is quite intermediate in pubescence.

9E. *OENOTHERA CONTORTA* var. *epilobioides* (Greene), n. comb.—*O. strigulosa* var. *epilobioides* Greene, Fl. Francisc., 216. 1891; *Sphaerostigma contortum* var. *Greenii* Small, Bull. Torr. Bot. Club 23:189. 1896; Nelson, BOT. GAZ. 40:58. 1905; *O. dentata* var. *cruciata* S. Wats., Proc. Am. Acad. 8:594. 1873, in part; *O. strigulosa* var. *cruciata* (S. Wats.) Greene, Fl. Francisc., 216. 1891, in part; *Sphaerostigma campestre* var. *minus* Small, Bull. Torr. Bot. Club 23:189. 1896, in part; Nelson, BOT. GAZ. 40:57. 1905, in part.

Large, commonly 2.5–4 dm. high, erect; stems mostly glabrate, but if pubescent, the hair is spreading (especially in plants from Central California); inflorescence often glandular; capsules long, 25–40 mm., sessile, slender, commonly beaked.

Type locality not stated; ranging from Southern Oregon to Northern Lower California in the interior valleys of the region west of the Sierra Nevada and reaching the coast in Southern California: OREGON, Grants Pass, *Henderson* in 1886 (S); Roseburg, *Howell* in 1887 (C, WS); Rogue River, *Leiberg* 4172 (US); CALIFORNIA, Redding, *Heller* 7888 (G); Burney, *Eastwood* 1046 (US); Yosemite, *Abrams* 4426 (G); Tracy, *Baker* 2775 (G); California, *Hartweg* 1733 (G); Poso Creek, *Hall & Babcock* 5012 (G); Santa Cruz, *Anderson* in 1864 (G); Tassajara, *Elmer* 3187 (S); Orcutt, *Jones* in 1926 (P); Santa Barbara, *Elmer* 4001 (G); Carpinteria, *Brewer* 276 (C, G); Topatopa Mts., *Abrams & McGregor* 155 (G); Inglewood, *Abrams* 3237 (G); Elizabeth Lake, *Hall* 3051 (C, G, P, S, W); Cajon Pass, *Parish* 4845 (C, S); San Bernardino Valley, *Parish* 5600 (W), 6935 (C); Moreno Valley, *Hall* 3842 (C); Riverside, *Hall* 2954 (C); Banning, *Jones* in 1903 (P); Kenworthy, *Munz & Johnston* 5466 (C, P); Idyllwild, *Spencer* 2201 (P); Hemet, *Baker* 4116 (C, G, P); Fallbrook, *Jones* in 1882 (P, W); Pala, *Munz* 10371 (P); Oak Grove, *Munz* 10381 (P); Warners Ranch, *Keck & McCully* 53 (P); Cuyamaca Mts., *Munz* 9792 (P); Palomar Mts., *Chandler* 5379 (C); Laguna Mts., *Munz* 9674 (P); Descanso, *Munz & Harwood* 7137 (C, P); Jacumba, *Munz* 8091 (P); Ramona, *Brandegge* in 1894 (C); Foster, *Spencer* 110 (G); Old Mission Dam, *Chandler* 5000 (C); LOWER CALIFORNIA, Hot Springs (Tia Juana), *Jones* in 1882 (C); 50 miles S. E. of Tecate, *Munz* 9548 (P); 9 miles from Tecate, *Munz* 9477 (P).

The name *epilobioides* is used for this variety since it seems to be the oldest one about which there is no uncertainty. The name *cruciata* is older, but seems applicable rather to a group of plants that are intermediate between *O. contorta* var. *epilobioides* and *O. dentata* var. *campestris*, than to *epilobioides* itself. If we apply the principle of selecting as type the first specimen cited in a description,

we should have to choose *Hartweg* 1733 which I refer to var. *epilobioides*, but this does not agree with my interpretation of what WATSON really meant by his *cruciata*. The *Hartweg* collection has petals less than 4 mm. long, while the description gives 4 mm. as the length and agrees with such plants as Oroville, *Heller* 11330 (C, G, S) and Antioch, *Rattan* in 1879 (S), which are small-flowered forms of *dentata*. The name *epilobioides*, as used in this paper, is rather inclusive and has a number of variations, such as (1) inflorescence glandular, *Munz & Harwood* 7137, *Chandler* 5379, *Munz* 9548, 10381; (2) inflorescence both glandular and with spreading hair, *Munz* 9477; (3) strigillose, *Munz* 10371, *Baker* 4116; (4) glandular and strigillose, *Munz* 9674, *Munz* 9792; and (5) spreading hairs only, *Munz* 8091. *Hall* 3051 and *Parish* 4845 both are from the edge of the desert region, are low, and resemble var. *typica*.

10. *OENOTHERA DENTATA* Cav., Ic. 4:67. pl. 398. 1795.—Annual, usually bushy, being freely branched from base; stems subdecumbent to ascending, occasionally subsimple and erect, slender, even capillary, 5–20 cm. high, with light-colored epidermis tending to exfoliate when old, hirsutulose to glabrate, or strigillose or glandular; leaves well distributed, mostly lance-linear, subsessile, often fascicled, pubescent to glabrous, remotely denticulate, acute, 5–30 (–50) mm. long, 1–4 (–7) mm. wide, gradually smaller as one passes up the stem, those of inflorescence reduced to leafy bracts; flowers few, in uppermost axils, not crowded; calyx tube obconic, glabrous within, glandular to strigillose without, 2–4 mm. long; calyx lobes lance-ovate, glandular or strigillose, 3–12 mm. long; petals bright yellow with or without red dots at base, suborbicular-obovate to -obcordate, 5–15 mm. long; filaments glabrous, yellow, distinctly of two lengths, the longer half to two-thirds the length of the petals; anthers oblong, glabrous, 1–2 mm. long, yellow; style glabrous, 6–15 mm. long, usually distinctly shorter than the petals; stigma 0.7–3 mm. broad; capsules linear, terete, somewhat torulose, straight or somewhat contorted, strigillose or glabrate, 2–4 cm. long, 0.5–1.0 mm. thick, with or without terminal beak; seeds brown, linear-obovoid, somewhat angled and flattened, minutely cellular-punctate, 0.5–1 mm. long.

The type of *O. dentata* came from “prope Coquimbo et Talcaguano, urbes Chilenses.” The original description and the plate agree fairly well with North American material except for the stigma, which is said to be “quadridum.” I have not had access to enough South American material to present any treatment including it, and must confine my attention here to that from North America.

## KEY TO VARIETIES

Petals 5-8 mm. long; capsules slender, about 0.5 mm. thick; seeds 0.5 mm. long

Stems short-villous in lower portion at least; inflorescence glandular; capsules with well defined beak; in California north of Santa Barbara County and Tehachapi . . . . . 10a. *O. dentata* var. *campestris*

Stems glabrate or with short appressed hair; inflorescence canescent-strigillose or glandular; capsules not distinctly beaked; Santa Barbara and Tehachapi to Elsinore and Hemet, California . . . . . 10b. *O. dentata* var. *parishii*

Petals 8-15 mm. long; capsules thicker, about 1 mm. in diameter; seeds about 1 mm. long; Western Mohave Desert into Nevada

10c. *O. dentata* var. *johnstonii*

10A. *OENOTHERA DENTATA* var. *CAMPESTRIS* (Greene) Jepson, Man. Calif., 685. 1925.—*O. campestris* Greene, Fl. Francisc., 216. 1891; *Sphaerostigma campestre* (Greene) Small, Bull. Torr. Bot. Club 23:189. 1896, in part; Nelson, Bot. Gaz. 40:57. 1905; apparently *O. torulosa* f. *mixta* Leveille and f. *permixta* Leveille, Monogr. Onoth., 180. 1905; and *Sphaerostigma campestre* var. *mixtum* (Levl.) Nelson, l. c.

Stems short-villous below with spreading hair; inflorescence glandular; petals 5-8 mm. long; capsules slender, 0.5 mm. thick, usually with well defined terminal beak; seeds 0.5 mm. long.

Type locality not given, but the description refers back to the *O. dentata* of Watson in Bot. Calif. 1:226. 1876, which is based on that of T. & G. Fl. N. Am. 1:510. Their plant was one collected in California by Douglas (G), and which must have come from near the coast. It agrees in pubescence and other characters with what I am including under var. *campestris*. The variety ranges in the San Joaquin and Sacramento valleys and sparingly from Antioch to Santa Barbara: CALIFORNIA, Lakeport, Baker in 1903 (C, P); Antioch, Baker 2807 (C, G, P, W); Modesto, K. Brandegee in 1917 (P); Clovis, Thompson in 1902 (P, S); Alcalde, T. S. Brandegee in 1891 (C); Lindsay, Munz 9084 (P); Greenhorn Range, Hall & Babcock 5037 (C, S); Poso Creek, Dudley 561 (S); Merced River, Bolander 4844 (G); San Antonio Creek, Dudley in 1895 (S); Nacimiento River, State Survey 550 (C); Santa Margarita fossil bed, Summers in 1886 (C, P, W). Baker 2807 has rather small flowers (petals 5-6 mm. long) and would be near Watson's var. *cruciata* which was discussed under the preceding species. The Brandegee collection from Alcalde and the Thompson one from Clovis approach var. *johnstonii* in the large flowers, but not in the pubescence of the stems.

One name that needs discussion is *Sphaerostigma campestre* var. *helianthemiflorum* (Levl.) Nelson, Bot. Gaz. 40:57. 1905. NELSON does not define his use of the variety, but one would infer that he refers to the large-flowered material that I am calling *johnstonii*. His discussion refers back simply to LEVEILLE's

description and plate (Monogr. Onoth., 178 and plate. 1905). The plate figures three collections, one of which, *S. B. and W. F. Parish* 1304, is var. *parishii*; the second, *Palmer* 136, I have not seen, but it may be var. *johnstonii*; the third is uncertain, but certainly not var. *johnstonii*. Furthermore, LEVEILLE's citation of specimens for his *O. torulosa* race *helianthemiflora* includes plants of var. *campestris* as well. So far as I can see, LEVEILLE meant this race to consist of all the large-flowered material that I refer to *dentata*, while *torulosa* itself was largely what I am calling *contorta*. The name *helianthemiflora*, then, is not based on any particular type or definite or restricted concept, and I feel under no obligation to use it for the large-flowered plants for which I here propose the name:

10B. *OENOTHERA DENTATA* var. *johnstonii*, n. var.—Stems glabrate or glandular-pubescent; inflorescence glandular-pubescent; leaves up to 6 mm. wide; flower large, petals 10–15 mm. long; calyx lobes 5–12 mm. long; capsules 1 mm. thick, not conspicuously beaked; seeds about 1 mm. long. (Flores magni; lobis calycis 5–12 mm. longis; petalis 10–15 mm. longis; capsulis 1 mm. crassis; seminibus circa 1 mm. longis.)

Type, near Mohave, *I. M. Johnston* on May 5, 1920 (Pomona College Herb. no. 8844); ranging over western half of Mohave Desert and north-eastward into Nevada: NEVADA, Nipton, *Jones* in 1907 (P); CALIFORNIA, Mill Creek Canyon, Panamints, *Coville & Funston* 759 (G, S); Shepherds Canyon, *Jones* in 1897 (P); Inyokern, *Bailey* in 1922 (P); Darwin, *Austin* 539 (C); Cameron, *K. Brandegee* in 1884 (C); Hinkley, *Jaeger* 125 (P); Barstow, *Munz & Keck* 7931 (P); Garlic Springs, *Munz & Keck* 7869 (P); Granite Wells, *Parish* 10117 (S); Randsburg-Rand, *K. Brandegee* in 1913, in part (C); Rosedale, *Abrams* in 1900 (S); Sunset, *Heller* 7728 (C, G, S); Devils Den, *Pierce* in 1922 (P); Tehachapi, *Brandegee* in 1884 (C), *Jones* in 1903 (P), *Munz* 8961 (P); Gormans Station, *Parish* 1973 (G, S); N. of Tejon Pass, *Munz* 9003 (P); Mohave, *Heller* 7762 (C, G, S); Tejon Pass, *Dudley & Lamb* 4442 (P, S); Willow Springs, *Munz* 10043 (P); Grapevine, *Newsom & Hilend* in 1927 (P); Elizabeth Lake, *Hall* 3066 (C), *Munz* 9989 (P); Oil City, *Heller* 7590 (C, G, S).

10C. *OENOTHERA DENTATA* var. *parishii* (Abrams), n. comb.—*Sphaerostigma campestre* var. *parishii* Abrams, Fl. Los Angeles, 272. 1904; *S. campestre* of Small, Bull. Torr. Bot. Club 23:189. 1896, in part.

Stems glabrate or with short appressed hair; inflorescence usually canescent, sometimes glandular; flowers as in var. *campestris*; capsules slender, 0.5 mm. thick, not distinctly beaked; seeds 0.5 mm. long.

Type locality near San Bernardino; range over western half of Mohave Desert and occasional in interior valleys from Santa Barbara to Los Angeles counties, more common from San Bernardino to Elsinore and Hemet: CALIFORNIA, Terra Bella, Tulare Co., *Abrams* 10855 (S); Caliente Creek, *Munz* 8989 (P); Mt. Pinos, *Hall* 6439 (C); Ft. Tejon, *Hall* 6276 (C); Mohave, *Jones* in 1903 (P); Lebec, *Jones* in 1927 (P); Pallett Creek, *Munz* 6904 (P); Acton, *Elmer* 3715 (G, P, S); Swartout Canyon, *Hall* in 1899 (C); Victorville, *Munz* 2557 (P, S); Barstow, *Hall & Chandler* 6849 (C, P); Daggett, *Hall* 6141 (C); Argus Mts. *Purpus* 5436 (G); Leastalk, *Parish* 10361 (S); Cajon Pass, *Munz* 4475 (P); San Bernardino Valley, *Parish* 7102 (C), in 1893 (C), in 1901 (S); Riverside, *Koethen* 16 (C); Hemet, *Baker* 4115 (C, P, S); Winchester, *Hall* 2417 (C, P, S, W); San Jacinto, *Jones* 3168 (C, P, W); Elsinore, *McClatchie* in 1892 (S).

Most of the material referred here is not glandular, but the following collections are: *Munz* 4475, *Hall* 6439, 6276, *Munz* 6904, *Elmer* 3715, and *Hall & Chandler* 6849.

11. *OENOTHERA GUADALUPENSIS* S. Wats., Proc. Am. Acad. 11:115. 1876.—Annual, erect, 8–10 cm. high, simple or branched from base, strigillose pubescent throughout; stems leafy; leaves oblanceolate, “sessile or the lowest attenuate into a petiole, obtuse or acutish, obscurely sinuate-toothed,” 2–3 cm. long, about 1 cm. wide; “flowers few, axillary”; calyx tube obconic, 2.5 mm. long, glabrous within; calyx lobes ovate, 2.5 mm. long; petals narrowly obovate, yellow, 2.5–3 mm. long; filaments glabrous, 1 to 2 mm. long; anthers rounded, scarcely 1 mm. long; style glabrous, 3.5 mm. long; stigma about 1 mm. broad; capsules strigose “oblong-pyramidal,” nearly straight, strongly angled, about 12 mm. long, 2.5 mm. in diameter; seeds “brown, smooth.”

Collected on Guadalupe Island by *E. Palmer* in 1875 (G); this, the type collection, is all that I have seen. It offers scarcely enough material to satisfy me as to whether *O. guadalupensis* is specifically distinct or whether it should be reduced to varietal rank under *O. hirta* Link.

12. *OENOTHERA HIRTA* Link, Enum. Hort. Berol. 1:378. 1821.—*Sphaerostigma hirtum* F. & M., Ind. Sem. hort. Petr. 2:22. 1835; *Oenothera micrantha* Hornem., ex Sprengel, Syst. 2:228. 1825; not *O. hirta* L., Syst. Ed. X, 998. 1760.

Annual, simple or several-stemmed, prostrate to ascending or erect, glabrate to hirsute, 5–60 cm. tall, leafy throughout, with stems pale to reddish, and with readily exfoliating epidermis; basal leaves forming a sort of rosette, linear-lanceolate to oblanceolate, pubescent

to pilose, almost entire, obtuse to acute, 20–100 mm. long, 4–15 mm. wide, gradually narrowed into equal or shorter petioles; lower cauline leaves shorter and broader, subsessile to short-petioled; upper cauline leaves sessile, lance-linear to lance-oblong to ovate-clasping, largely secund and often undulate, gradually reduced up the stem; flowers in axils of even lowest leaves; calyx tube 1.5–5 mm. long, glabrate to pubescent within, glabrate to pilose without; calyx lobes lanceolate, 1–7 mm. long, glabrate to pilose; petals yellow often drying green, 1.5–7 mm. long, obovate to obcordate, emarginate, rounded or salient-toothed; stamens of two lengths, the longer ones about two-thirds length of petals, shorter ones about half the length; anthers oblong to linear-oblong, 0.5–1.0 mm. long; style equalling longer stamens, glabrate; stigma 0.5–1 mm. broad; capsules curved or contorted, quadrangular, glabrous to pubescent, 1–2 mm. thick, 10–25 mm. long, gradually attenuate upward and usually beaked; seeds brown, obovoid, finely cellular-pitted, ca. 1 mm. long.

#### KEY TO VARIETIES

Flowers small, petals 2–3 (–4) mm. long; stems and leaves hirsutulous or villous, but not pallid

Stems semi-prostrate; cauline leaves oblong-lanceolate, obtuse, sessile but not clasping; largely coastal. . . . . 12a. *O. hirta* var. *typica*

Stems erect or ascending; cauline leaves oblong-ovate to broadly ovate, acute, with subcordate clasping base; largely of the interior valleys

12b. *O. hirta* var. *jonesii*

Flowers larger; petals 3–7 mm. long; foliage either glabrate, or pallid with close whitish pubescence

Plants glabrate; stems often simple, erect; interior valleys of costal drainage

12c. *O. hirta* var. *ignota*

Plants pallid with whitish pubescence; stems mostly several from near the base, spreading; deserts. . . . . 12d. *O. hirta* var. *exfoliata*

12A. *OENOTHERA HIRTA* Link var. *typica*, n. nom.—*O. hirta* Link, l. c., *Sphaerostigma hirtum* F. & M., l. c.; *Oenothera micrantha* Hornem., in Sem. hort. bot. hafn., 16. 1822, without description; ex Sprengel, Syst. 2:228. 1825; *Holostigma micranthum* Spach, Nouv. Ann. Mus. Par. 4:335. 1835; *Sphaerostigma micranthum* Walp., Rep. 2:77. 1843; *O. cheiranthifolia* var. *contorta* of Levl., Mon. Onoth., 216. 1905, in large part.

Semi-prostrate; basal leaves oblanceolate; cauline leaves oblong-

lanceolate, sessile, obtuse, undulate, denticulate, commonly 5-7 mm. wide; flowers small; petals 2-4 mm. long; capsules short, 12-20 mm. long.

Type locality, California, probably from the northern coast; ranging largely along the coast from Bodega Point to Lower California: CALIFORNIA, Antioch, *Baker* 2808 (C, G, P, W); San Francisco, *Curran* in 1894 (S), *Davy* in 1896 (C); Mt. Diablo, *Abrams* 8022 (S); Del Monte, *Heller* 6659 (C, G, P, S); San Antonio Mission, *Dudley* in 1895 (S); Paso Robles, *Barber* in 1899 (C); Pismo Beach, *Abrams* 6513 (S); Santa Rosa Island, *Brandegee* in 1888 (C); Buellton, *Munz* 10289 (P); Santa Susanna Mts., *Brewer* 221 (C); Santa Monica Mts., *Crawford & Hiatt* in 1916 (P, S); Inglewood, *Abrams* 3232 (C, G, P, S); Ballona Harbor, *Abrams* 1192 (P, S); Claremont, *Robinson* 93 (P); Temescal Mts., *Brewer* 155 (C); Fallbrook, *Jones* in 1882 (P, C, W); San Diego, *Jones* in 1882 (P); Bird Rock, *F. E. & E. S. Clements* 184 (C, G), 185 (C, G); Jamul, *Stokes* in 1895 (S): LOWER CALIFORNIA, San Pedro Martir, *Brandegee* in 1889 (C); San Telmo, *Brandegee* in 1893 (C); San Quentin Bay, *Palmer* 617 (C, US), 656 (US); San Antonio Canyon, *Ballou* 108 (P).

The varieties in this species are not any too well marked and intergradation is frequent. The leaf shape and habit of this variety and of var. *jonesii* are so variable as to make many plants difficult of determination; the following may be cited as intergrades: Mt. Hamilton-Livermore Road, *Bacigalupi* in 1923 (S); Mill Valley, *Bioletti* in 1892 (W); Santa Barbara, *Brewer* 324 (C); Bardsdale, Ventura Co., *Hall* 3241 (C); Santa Monica Mts., *Abrams* 1273 (S).

I have a photograph of the type of *O. micrantha* Hornem. and have had access to authentic material of *O. hirta* Link (NY). CHRISTENSEN of Copenhagen has kindly given me information showing the identity of these two species. Since LINK's name is the older, and since LINNAEUS' *O. hirta* is universally regarded as a *Jussiaea*, I here take up the name *hirta* for the plant so long called *micrantha*.

12B. *OENOTHERA HIRTA* var. *JONESII* Leveille, Mon. Onoth., 213. 1905.—*Sphaerostigma micranthum* var. *jonesii* (Levl.) Nelson, BOT. GAZ. 40:59. 1905; *Oenothera hirtella* Greene, Fl. Francisc., 215. 1891.; *Sphaerostigma hirtellum* (Greene) Small, Bull. Torr. Bot. Club 23:190. 1896; Nelson, BOT. GAZ. 40:59. 1905; *S. arenicolum* Nelson, BOT. GAZ. 40:58. 1905.

Erect or ascending, densely villous-pubescent throughout; cauline leaves oblong-ovate to broadly ovate; acute, often sessile, with subcordate clasping base; flowers as in *typica*.

Type not stated, but description refers to plate showing *Jones* 2231 from Santa Cruz, California, which I take to be the type and not *Hansen* 543 as cited by NELSON, although LEVEILLE gives the latter first in the list of collections

belonging here. This variety tends to occur on hills and lower mountains of the interior valleys: CALIFORNIA, Anderson Valley, Mendocino Co., *Purdy* in 1882 (G); Lakeport, *Tracy* 1701 (C); Alder Springs, Glenn Co., *Heller* 11443 (G, S); Knoxville, Napa Co., *Jepson* in 1892 (C); Mt. Diablo, *Bioletti* in 1894 (C); Santa Cruz, *Jones* 2231, type coll. (P); Loma Prieta, *Hitchborn* in 1920 (S); Los Gatos, *Heller* 7337 (C, G, S, W); Pajaro Hills, *Chandler* 436 (C); Monterey, *Elmer* 3192, type coll. *S. arenicolum* (S); Santa Lucia Mts., *Abrams* 7410 (S); Santa Ynez Mts., *Elmer* 3943 (G, P, S); Drytown, Amador Co., *Hansen* 543 (C, P, S); Erskin Creek, *Purpus* 5099 (C); Bouquet Canyon, *Munz* 6928 (P); Santa Monica, *Munz & Harwood* 3928 (P); San Gabriel Canyon, *Munz* 9427 (P); Claremont, *Chandler* in 1897 (C); Victorville, *Parish* 10624 (S); Strawberry Valley, San Jacinto Mts., *Wilder* 954 (C); Poppet Flat, *Munz & Johnston* 8847 (P); Santa Rosa Mts., *Munz* 5940 (P); Santiago Peak, *Munz* 7064 (C, P); Elsinore, *Munz* 5086 (P); Dripping Spring, *Munz* 9833 (P); Henshaw Dam, *Munz* 8333 (P); Santa Ysabel, *Munz* 9809 (P); Campo, *Abrams* 3558 (C, G, P, S); Barrett Dam, *Munz* 8002 (P); Ocean Beach, dist. C. F. *Baker* 1645 (C, G, P, W); LOWER CALIFORNIA, 13 miles S. E. of Tecate, *Munz* 9516 (P); San Quentin, *Brandegee* in 1889 (C); Cardon Grande, *Brandegee* in 1889 (C); Todos Santos, *Orcutt* in 1882 (C); Rosario, *Orcutt* in 1886 (C).

In the mountains of Southern California, occurring in the same fields with the usual form, grows a plant with the petals variously toothed, commonly having a long central tooth with a sinus and shorter tooth on each side. This variation, which is not at all constant, may be designated as *O. hirta* var. *jonesii* forma *reedii* (Parish), n. comb. (*Sphaerostigma bistorta* var. *reedii* Parish, *Muhlenbergia* 3:60. 1907; *Oenothera micrantha* var. *reedii* Jepson, *Man. Calif.*, 684. 1925; *Sphaerostigma hirtellum* var. *montanum* Davidson, *Muhlenbergia* 3:108. 1907, in part). Here may be cited PARISH'S type, Waterman Road, San Bernardino Mts., *Parish* 5794 (C, G); Poppet Flat, *Munz & Johnston* 8846 (P); Oak Grove trail to Palomar Mt., *Munz* 10390 (P).

12C. *OENOTHERA HIRTA* var. *ignota* (Jepson), n. comb.—*O. micrantha* var. *ignota* Jeps., *Man. Calif.*, 684. 1925.

Glabrate; stems often simple, erect; calyx glabrate; flowers larger; petals 5–7 mm. long.

Type locality, Jurupa Hills, near Riverside, California: CALIFORNIA, Polasky, Madera Co., *Heller* 8166 (C, G, S); Leonis Valley, *Davy* 2620 (P); Kern River Canyon, G. D. *Abrams* in 1900 (S); Ft. Tejon, *Davy* 2375 (C); Mt. Piños, *Dudley & Lamb* 4698 (S); Saugus, *Munz* 10013 (P); Antelope Valley, *Davy* 2529 (P); North Baldy Mt., *Abrams & McGregor* 579 (S); Mt. Wilson, *Grant* in 1900



(S); San Gabriel Canyon, *Munz* 9461 (P); San Dimas Canyon, *Munz & Harwood* 3692 (P, W); San Bernardino, *Parish* in 1901 (S), 3670 (C, G); Waterman Canyon, *Jones* in 1926 (P); Colton, *Jones* in 1882 (P); Slover Mt., *Parish* 73 (C, G, S); Jurupa Hills, *Wilder* 90, type coll. (C), *Johnston* in 1920 (P); Corona, *Munz & Harwood* 3373 (P, S); Elsinore, *McClatchie* 77 (S); San Jacinto Valley, *Reinhardt* in 1897 (C); Potrero Grade, *Munz* 9468 (P); Laguna Mts., *Munz* 9677 (P); Fallbrook, *Jones* in 1882 (C, P, W): LOWER CALIFORNIA, 50 miles S. E. of Tecate, *Munz* 9559 (P).

The var. *ignota* is another variation but a real entity. Intergrades with var. *typica* are as follows: Wilson Trail, *Abrams* 1505 (S); Fallbrook, *Abrams* 3326 (C, P, S); Saugus, *Davy* in 1901 (C); Jamesburg, Monterey Co., *Bacigalupi* 1143 (S). In these plants the flowers are rather large for var. *typica*, having petals 4-5 mm. long; the leaves are lanceolate and somewhat narrow and hairy for var. *ignota*. Another set of intergrades is with var. *jonesii*; they tend to have flowers large for *jonesii*, and leaves wide, and to be rather hairy for var. *ignota*. Oak Grove Trail to Palomar Mt., *Munz* 10398, 10400 (P); Arrowhead Lake, *Hilend* in 1923 (P); Acton, *Barber* 221 (C); Topango, *Munz & Harwood* 3968 (P); Jacumba, *McGregor* 112 (S). Another series intergrades with the var. *exfoliata*, tending to have the pallid condition suggesting the latter variety, but the erect habit of growth of *ignota*: Idyllwild Road from Banning, *Munz* 8142 (P); Fullers Mills Mts., *Hall* in 1901 (C); Borrego Spring, *Jones* in 1906 (P); mesas west of Independence, *Hall & Chandler* 7300 (C, P, W).

12D. *OENOTHERA HIRTA* var. *exfoliata* (Nelson), n. comb.—*Sphaerostigma micranthum* var. *exfoliatum* Nelson, BOT. GAZ. 40: 59. 1905; *Oenothera micrantha* var. *abramsi* (Macbr.) Jepson, Man. Calif., 684. 1925; *O. abramsi* Macbr., Contr. Gray Herb. 65: 41. 1922; *Sphaerostigma pallidum* Abrams, Bull. Torr. Bot. Club 32: 539. 1905.

Plant pallid with dense whitish pubescence; stems usually several from near the base, spreading; flowers rather large; petals 3-6 mm. long.

Type locality, Colorado Desert; confined to the deserts and desert borders: CALIFORNIA, Antelope Valley, *Hall* 3052 (C); north side of Cajon Pass, *Munz, Harwood, & Johnston* 4075 (P); Cactus Flat, *Munz* 10518 (P); Providence Mts., *Munz, Harwood, & Johnston* 4017 (P, W), *Munz & Harwood* 3442 (C, P); Quail Springs, *Munz & Johnston* 5277 (P); Cabazon, *Munz, Street, & Williams* 2360 (P, S); Palm Springs, *Johnston* 1110 (P, S, US), *Spencer* 1452 (P, G); Indian Wells, *Munz & Keck* 4978 (P); Coyote Canyon, *Hall* 2791 (C, US); San Felipe Valley, *Keck & McCully* 70 (P); Mountain Spring, *Mearns* 3037 (US), *Peirson* 2879 (P); Cholla Ranch, *Jones* in 1906 (P): ARIZONA, Congress, *Orcutt* in 1896 (C); Chloride, *Jones* in 1903 (P); Yucca, *Jones* in 1884 (P); Skull Valley, *Jones* in 1903 (P); Tempe, *Ganong & Blaschka* in 1892 (G); Verde River, *Smart* 146 (G).

The species has only one good variety on the deserts, the pallid var. *exfoliata* with its rather large flowers. It and the var. *ignota* stand between *O. hirta* Link and *O. bistorta* Nutt., so far as flower size is concerned. As a varietal name *exfoliata* has priority over any other given to the desert plant. There are two forms: one with narrow almost linear leaves, *Spencer* 1452, *Johnston* 1110, *Munz & Harwood* 3442; and a less common one, with leaves broader and suggesting the var. *jonesii*, *Jones* at Skull Valley and *Yucca*, and *Munz* 10518.

13. *OENOTHERA BISTORTA* Nutt., ex T. and G., Fl. N. Am. 1:508. 1840.—Annual, occasionally simple, but usually with several prostrate or ascending stems, these strigose to villous, light in color, often tinged reddish, with exfoliating epidermis, rather slender, 5–80 cm. long; leaves pubescent to thinly pilose, denticulate or subentire; those of the basal rosette 3–7 cm. long, 0.3–0.5 cm. wide, linear-oblongate, narrowed into petioles 1–4 cm. long; cauline leaves often secund, shorter and wider than basal ones; uppermost lanceolate to lance-oblong or lance-ovate, acute to obtuse, subsessile to cordate-clasping, 1–3 cm. long, 0.5–2 wide; flowers in axils, only a few in anthesis at once; calyx tube obconic, 3–5 mm. long, glabrate to pubescent within, villous without; calyx lobes villous, lanceolate, 7–10 mm. long; petals yellow, often drying greenish, with or without dark spot at base, suborbicular to obovate to obcordate, 8–14 mm. long; filaments glabrous, distinctly of two lengths, longer ones half to two-thirds the length of the petals; anthers 2.5–3 mm. long, yellow; style glabrate to finely pubescent, 7–11 mm. long, slightly exceeding stamens; stigma 1–1.5 mm. broad; capsule curved or contorted, 1.5–2.5 mm. thick, 12–40 mm. long, with or without beak, pubescent, somewhat quadrangular; seeds brown, obovoid, about 1 mm. long, finely cellular-pitted.

#### KEY TO VARIETIES

Foliage in general, green; plants of coastal drainage

Capsule short, 12–15 (–20) mm. long, sharply quadrangular, usually 2–2.5 mm. thick, with beak lacking or not exceeding 4–5 mm.; strictly coastal, largely in San Diego region. . . . . 13a. *O. bistorta* var. *typica*

Capsule longer, 20–40 mm., more slender, 1.5–2 mm. thick, and with beak 3–10 or more mm. long; rather generally distributed in coastal drainage of Southern California. . . . . 13b. *O. bistorta* var. *veitchiana*

Foliage pallid with short appressed hair; desert plants

13c. *O. bistorta* var. *hallii*

13A. *OENOTHERA BISTORTA* Nutt. var. **typica**, n. nom.—*O. bistorta* Nutt., l. c.; Watson, Proc. Am. Acad. 8:592. 1873; *Sphaerostigma bistortum* (Nutt.) Walp., Rep. 2:77. 1843; Small, Bull. Torr. Bot. Club 23:190. 1896; Nelson, Bot. Gaz. 40:59. 1905; probably *Holostigma bottae* Spach, Nouv. Ann. Mus. Paris (III) 4:336. 1835; *Oenothera spiralis* var. *linearis* Jepson, Man. Calif., 684. 1925.

Capsule short, 12–15 (–20) mm. long, sharply quadrangular, usually 2–2.5 mm. thick, with beak lacking or not over 4 or 5 mm. long.

Type locality, San Diego; ranging along the very coast from Ballona Harbor and Catalina Island to San Diego and northern Lower California: CALIFORNIA, Catalina Island, *Trask* in 1896 (C); Ballona Harbor, *Abrams* 300 (P); Canada Salada, *Peirson* 3411 (P); Laguna Beach, *Crawford* in 1916 (P); Sunnyside, *Hall* 3907 (C), 3908, type coll. of *O. spiralis* var. *linearis* (C); La Jolla, *F. P. & E. S. Clements* 181 (C, G), 182 (G), *Chandler* 5138 (S); Soledad, *Jones* in 1882 (P); Torrey Pines Park, *Munz* 7956 (P); Del Mar, *Brandegee* in 1894 (C); San Diego, *Nuttall*, type of *O. bistorta* (G), *K. Brandegee* in 1906 (C, P), *Jones* 3058 (P, W), San Diego River near the mission, *Abrams* 3412 (C, G, P, S); Sweetwater Valley, *Stokes* in 1895 (S); Point Loma, *Eastwood* 2864 (G); San Pasqual, *Thurber* 602 (G); LOWER CALIFORNIA, Hot Springs, now Tia Juana, *Jones* in 1882 (C, P, W).

Intergrades between this variety and the var. *veitchiana* are as follows: Ballona, *Abrams* 1232 (P, S) and Playa del Rey, *Grant* in 1904 (W), San Diego, *C. F. Baker* distribution 3395 (C, G, P), all of which have capsules quite intermediate in character.

13B. *OENOTHERA BISTORTA* var. **VEITCHIANA** Hook., Bot. Mag. 84:pl. 5078. 1858.—*O. bistorta* var. *veitchiana* in Watson, Proc. Am. Acad. 8:593. 1873; *Sphaerostigma veitchianum* (Hook.) Small, Bull. Torr. Bot. Club 23:191. 1896; *S. bistortum* var. *veitchianum* (Hook.) Nelson, Bot. Gaz. 40:59. 1905.

Capsule more slender, 1.5–2 mm. thick, and longer, 20–40 mm. with the beak 3–10 or more mm. long.

Type locality, near San Gabriel, Los Angeles County, California; ranging rather generally through the interior valleys and hills of the coastal drainage of Southern California as well as along the coast itself: CALIFORNIA, South Fork of the Kern, *Purpus* 5718 (C); Ft. Tejon, *Xantus* in 1857–8 (G); Springville, *Davy* in 1901 (C); Moorpark, *Abrams* 10129 (P, S); Ojai, *Peckham* in 1866 (US); Santa Clara River, Ventura Co., *Gray* in 1885 (G); Bardsdale, *Hall* 3241c (C), 3241 b (C), 3241a (C); Saugus, *K. Brandegee* (C), *Munz* 10018 (P); San Fernando, *Munz* 9383 (P); Pasadena, *Grant* in 1905 (W); Monrovia, *Baker* 4171

(G, P); Claremont, *Munz* 2063 (P); Hesperia, *Shaw*, et al. in 1917 (P); Colton, *Jones* in 1882 (P); San Bernardino, *Parish* 3659 (C, G); Redlands, *Billings* in 1924 (S); Highlands, *Spencer* 1111 (G, P); Hemet, *Baker* 4118 (C, G, P, S); Dripping Springs, *Munz* 9838 (P); Pala, *Munz* 10370 (P); Fallbrook, *Munz* & *Harwood* 3894 (P); San Diego, *K. Brandegee* in 1906 (C); Julian, *Hall* in 1925 (P).

There is considerable intergradation between this plant and the inland forms of the preceding species (*O. hirta* Link). The principal definite character separating them is flower size, and that, especially in depauperate and late season plants, is none too constant. *Munz* & *Harwood* 3894a (P) from Fallbrook, *Hall* 429 from Winchester (C), and *Abrams* 2791 from Fredalba (S) are all examples of plants with small flowers, petals 4–5 mm. long, and except in habit, suggest *O. hirta* var. *ignota*. Usually in pressed specimens the flowers of *O. bistorta* have the petals spread, while in *O. hirta* var. *ignota* they do not.

13C. OENOTHERA BISTORTA var. HALLII (Davidson) Jepson, Man. Calif., 685. 1925.—*Sphaerostigma hallii* Davidson, *Muhlenbergia* 3:107. 1907.

Foliage pallid with short appressed hair.

Type locality, Banning; CALIFORNIA, Southern California, *Parry* & *Lemmon* 126 (G); Banning, *Hall* 446, type coll. (C), *Munz*, *Street*, & *Williams* 2363 (P, S.), *Jones* in 1903 (P); Indio, *McGregor* 707 (S); Warners Hot Springs, *Eastwood* 2592 (G). This rather uncommon variety corresponds, among the plants belonging to this species, to the var. *exfoliata* for the pallid desert plants of *O. hirta*.

14. OENOTHERA CHEIRANTHIFOLIA Hornem., ex Spreng., Syst. 2:228. 1825.—*Holostigma cheiranthifolium* (Hornem.) Spach, Nouv. Ann. Mus. Paris (III) 4:335. 1835; *Chamissonia cheiranthifolia* (Hornem.) Raim., in Engl. & Prantl, Nat. Pfl. Fam. 3:Abt. 7, 217. 1893.

Annual to perennial, with several prostrate to decumbent wiry or tough stems "radiating from a central rosette crowning the taproot," these 1–6 dm. long; plant rarely glabrous, usually closely white-pubescent to hirsute; leaves thick, those of rosette oblanceolate, 1–7 cm. long, 0.5–1.5 cm. wide, obtuse, subentire to remotely serrulate, narrowed into petioles 1–2 cm. long; lower cauline leaves lance-oblong to oblance-oblong, subsessile to short-petioled, obtuse, subentire to remotely denticulate, 2–4 cm. long, 0.5–1.5 wide; upper cauline leaves sessile, oblong-ovate to orbicular-ovate, obtuse, subentire, 0.5–2.5 cm. long, 0.5–2 cm. wide; flowers single in axils, mostly not near the base of the stems; calyx tube obconic, 2.5–7 mm. long, glabrous or glabrate within; calyx lobes lanceolate, 4–15 mm. long;

petals bright yellow, with or without reddish spots at base, usually drying green, sometimes pinkish, obovate, 7-22 mm. long and equally wide; filaments glabrous, 3.5-6 mm. long or 4.5-8 mm. long; anthers glabrous, linear, 1-2 mm. long; style usually glabrous, 8-22 mm. long; stigma 1-1.5 mm. broad; capsule coiled, distinctly quadrangular, short-beaked or not at all beaked, glabrous or pubescent, 12-22 mm. long; seeds blackish-brown, obovoid, 1 mm. long, minutely cellular-pitted.

#### KEY TO VARIETIES

Plant pubescent throughout

Petals 5-9 mm. long; plant grayish pubescent; along coast from Point Conception, California northward... 14a. *Oenothera cheiranthifolia* var. *typica*

Petals 10-22 mm. long; plant silvery; Point Conception southward

14b. *O. cheiranthifolia* var. *suffruticosa*

Plant glabrous throughout; rare, San Miguel Island and coast of Monterey County..... 14c. *O. cheiranthifolia* var. *nitida*

14A. *OENOTHERA CHEIRANTHIFOLIA* Hornem. var. *typica*, n. nom.—*Oenothera cheiranthifolia* Hornem., l. c.; Wats., Proc. Am. Acad. 8:592. 1873; *O. spiralis* Hook., Fl. Bor. Am. 1:213. 1833; Leveille, Monogr. Onoth., 220. 1905, in part; *Holostigma spirale* (Hook.) Spach, Nouv. Ann. Mus. Paris (III) 4:336. 1835; *Sphaerostigma spirale* (Hook.) F. & M., Ind. Sem. Hort. Petrop. 2:50. 1835; Small, Bull. Torr. Bot. Club 23:190. 1896; Nelson, Bot. Gaz. 40:60. 1905; *Holostigma cheiranthifolium* (Hornem.) Spach, l. c.; *Sphaerostigma cheiranthifolium* (Hornem.) F. & M., l. c.; *Chamissonia cheiranthifolia* Raim., in Engl. & Prantl., l. c.; *Agassizia cheiranthifolia* (Hornem.) Spach, Hist. Nat. Veg. Phaner., 4:348. 1825.

Plants grayish-pubescent throughout; flowers rather small, petals 5-9 mm. long.

Type locality, California, collected by WORMSKIOLD. In the original description, the type locality is given as Chile, a statement that has caused much confusion. At the Botanical Museum at Copenhagen there are three sheets, one with the following in HORNEMANN's handwriting, "Ex hort. semina e California mis. WORMSKIOLD," the second indicating that it came from WORMSKIOLD in 1820, and the third from "hb. Berol." On none of these does the name Chile appear, according to Dr. H. M. HALL of the Carnegie Institution, who kindly examined them for me. In addition to photographs and fragments from CHRISTENSEN of Copenhagen, I have seen a sheet at the Gray Herbarium from "Hort. Hamburg, Wormskiold." *O. cheiranthifolia* is a strand plant. The following

material is representative of the var. *typica*—"N.W. Coast" of America: *Menzies*, type of *O. spiralis*, photo of Kew specimen (P): OREGON, Port Orford, *Peck* 8506 (G); Gold Beach, Curry Co., *Hoyt* 76 (S); Chitco, *Howell* in 1884 (G); Sunset Cove, Coos Co., *Abrams & Benson* 10595 (S, W): CALIFORNIA, Eureka Peninsula, *Dudley* in 1899 (S); Clam Beach, *Munz* 9891 (P); Ocean Beach, *Tracy* 1263 (C); Samoa, *Tracy* 3017 (C, G, S); Ft. Bragg, *Duncan* 236 (S, W); Bodega Point, *Eastwood* 4832 (G); Oakland, *Jones* 2371 (P); San Francisco, *Baker* 697 (C, G, P); Del Mar, *Hichborn* 322 (S); Del Monte, *Heller* 6655 (C, G, P, S, W); Morro, *Munz* 9240 (P); Santa Rosa Island, *Brandegee* in 1888 (C); Santa Barbara Island, *Hemphill* (C); Surf, *Elmer* 3909 (G, P, S).

While flower size and, to a less extent, character of pubescence distinguish var. *typica* and var. *suffruticosa*, some plants are quite intermediate and difficult to place definitely. Examples are from Playa del Rey, *Grant* in 1904 (S) with small flowers, and from Santa Barbara, *Brandegee* in 1889 (C).

14B. OENOTHERA CHEIRANTHIFOLIA var. SUFFRUTICOSA S. Wats., Proc. Am. Acad. 8:592. 1873.—*O. viridescens* Hook., Fl. Bor. Am. 1:214. 1833; *Sphaerostigma viridescens* (Hook.) Walp., Rep. 2:77. 1843; Small, Bull. Torr. Bot. Club 23:190. 1896; *Oenothera spiralis* var. *viridescens* (Hook.) Jeps., Man. Calif., 684. 1925; *Sphaerostigma spirale* var. *viridescens* (Hook.) Nelson, Bot. Gaz. 40:60. 1905; *S. cheiranthifolium* var.  $\beta$ , F. & M., Ind. Sem. Hort. Petrop. 2:50. 1835; *S. spirale* var. *clypeatum* (Levl.) Nelson, Bot. Gaz., l. c.; *Oenothera virescens* of Greene, Fl. Francisc., 214. 1891.

Foliage silvery; perennial and usually suffrutescent; flowers large; petals 13–22 mm. long.

Type locality, California (*Coulter* 153, probably from near Santa Barbara or to the south); ranging along the strand from Santa Barbara to northern Lower California: "N. W. America:" *Menzies*, photo of type of *O. viridescens* (P): CALIFORNIA, Santa Barbara, *Elmer* 4018 (G, P, S); Carpinteria, *Hall* 3169 (C); Ventura, *Abrams* 10144 (P, S); Hueneme Beach, *Munz* 9393 (P); Santa Monica, *Barber* 24 (C); Redondo, *Grant* 1238 (W); Playa del Rey, *Grant* E1235 (W); Seal Beach, *Kendall* in 1920 (P); Ballona Harbor, *Abrams* 1679 (P, S); Balboa, *Abrams* 6576 (S); Laguna Beach, *Peirson* 4670 (P); Del Mar, *Brandegee* in 1894 (C); Oceanside, *Abrams* 6588 (S); La Jolla, *F. E. & E. S. Clements* 178, 179, 180 (C, G); San Diego, *Nuttall* (G), *Jones* in 1882 (P), *Wright* 52 (C); Coronado, *Chandler* 5090 (S), *Spencer* 112 (G, P): LOWER CALIFORNIA, Tia Juana, *Diehl* 214 (P); Ensenada, *Brandegee* in 1899 (C); San Quentin, *Ballou & Canby* in 1925 (P); Socorro, *Brandegee* in 1889 (C).

14C. OENOTHERA CHEIRANTHIFOLIA var. *nitida* (Greene), n. comb.—*O. nitida* Greene, Pittonia 1:70. 1887; *Sphaerostigma nili-*

*dum* (Greene) Small, Bull. Torr. Bot. Club 23:190. 1896; Nelson, BOT. GAZ. 40:58. 1905; *Oenothera spiralis* var. *nitida* (Greene) Jepson, Man. Calif., 684. 1925.

Flowers like those of var. *typica*, with petals 5-8 mm. long; plants glabrous throughout.

Type locality, San Miguel Island: CALIFORNIA, San Miguel Island, *Greene* in 1886, type coll. (C); between Castroville and Monterey, *K. Brandegee* in 1908 (C, G, W); Monterey County, *K. Brandegee* in 1889 (S); Seaside, near Del Monte, *Heller* 6654 (C, G, P, S, W), *K. Brandegee* (C); Del Monte, *Coleman* in 1908 (S). While the aspect of a wholly glabrous plant is quite different from that of a very hairy one, I agree with JEPSON that this is deserving of varietal rank only. The peculiar blunt leaves, habit of growth, and other characters are entirely those of *O. cheiranthifolia*. A collection made at Del Monte, by *Patterson & Wiltz* in 1907 (S) is intermediate, being sparsely pubescent.

#### EXCLUDED AND DOUBTFUL SPECIES

1. *Oenothera alata* Raf., Fl. Ludov., 95. 1817, placed in *Sphaerostigma* by Seringe, D.C., Prodr. 4:46. 1828; apparently to be referred to *Jussiaea*.

2. *Sphaerostigma andinum* var. *minutum* Nelson, BOT. GAZ. 40:56. 1905, is *Gayophytum caesium* T. & G., so far as I can tell from the depauperate plants which constitute the type; in any case, it is a *Gayophytum*.

3. *Sphaerostigma angelorum* (S. Wats.) Nelson, BOT. GAZ. 40:62. 1905, belongs to *Oenothera*, subgenus *Eulobus*.

4. *Sphaerostigma pterospermum* (S. Wats.) Nelson, l. c., 63, is in the subgenus *Chylismia*.

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# RESPIRATION OF THE SOY BEAN<sup>1</sup>

A. L. HAFENRICHTER

(WITH ELEVEN FIGURES)

## Introduction

BARNES (2) called attention to the overemphasis which was being placed, especially by botanists, upon the gaseous exchange of respiration. He pointed out the importance of a knowledge of the energy relations of this function, which can be attained only when more is known concerning the materials which furnish the energy and the specific manner in which they furnish it under the various conditions of the environment. This energy is derived, in the higher plants especially, from the organic compounds stored within the cells. It is liberated by hydrolysis and oxidation, and is believed by some to be dependent upon enzymatic reactions. That the carbohydrates and fats are the compounds which furnish the greater part of this energy is recognized by all physiologists. The proteins may furnish energy under certain conditions, but in the light of existing knowledge the amount is never very large. The proteins give by-products, however, which by their presence within the plant may exert a considerable influence upon the rate of metabolism of the carbohydrates and fats.

It has been known for a long time that the stored food materials of seeds undergo a series of changes during germination and development, and that these changes cause qualitative and quantitative differences in the materials subject to metabolism. The manner in which these qualitative and quantitative differences may affect respiration under various conditions has not been fully investigated, nor is it known whether or not the required amount of energy under

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any given set of conditions is always supplied by a definite metabolic process. It has been demonstrated by animal physiologists, in experiments on metabolism, that it is possible to maintain the same energy relations by substituting qualitatively different foods, provided the caloric values remain constant. Should such substitution be found to occur in higher plants, it would be of importance to know whether it follows definite laws, and in what way it is related to external conditions and the economy of the organism. In this respect it has been shown that some of the lower plants may use only one of two closely related carbohydrates when grown under certain conditions; further, that such plants may utilize different amounts of one food in the presence of different quantities of another. This seems to emphasize the possibility that external as well as internal conditions may determine the kind of respirable materials utilized by the plant as a source of energy.

### Historical review

During the germination of seedlings changes are constantly taking place in the various reserve food materials. Since the rate of change varies as development proceeds and is different for each of the several reserves, it would follow that there is a constant alteration in the nature and amount of respirable materials present at any one time. A similar variation would occur in those by-products of metabolism which are not directly respirable, but indirectly exert an influence. From a study of the literature bearing upon this question, it appears that the original composition of the seed may affect these relations.

PRIANISCHNIKOW (29) grew *Vicia faba* in distilled water in the dark for forty days. At ten-day intervals he made chemical analyses to determine the changes the reserve foods had undergone. The original seed contained high percentages of protein and starch, but little fatty material. As development progressed there was a decrease of protein-nitrogen, accompanied by an increase in amid- and asparagin-nitrogen. The most rapid change occurred during the first ten days. The same rate of change was noted for the decrease in starch content, whereas soluble carbohydrates increased slightly during the first period and thereafter decreased gradually. The ether

extract, although small at all times, varied as did the soluble carbohydrates. DETMER (4) made similar analyses with *Cannabis sativa*, a seed containing high percentages of fat and protein but no starch. The fat decreased rapidly during the first seven days and more slowly the following three days. After seven days considerable starch was found, but much of this had disappeared at ten days. The amount of protein remained essentially the same, and at no time were soluble carbohydrates found. FRANKFURT (7) obtained much the same result with *Helianthus annuus*, which has the same relative composition, but reported an increase in soluble carbohydrates and a decrease in protein-nitrogen not obtained by DETMER.

Comparative studies of the protein metabolism of *Vicia faba*, *Pisum sativum*, and *Lupinus luteus* were made by PRIANISCHNIKOW (30). The composition of *P. sativum*, according to SCHULZE, STIEGER, and MAXWELL (33), is very similar to that of *Vicia faba*, while *L. luteus* contains a high percentage of protein, considerable fat, but no starch. In all of these protein-nitrogen decreased, at first gradually, then rapidly to a maximum, and after this more slowly. However, at no time in their development was the rate of decrease the same in any of the plants studied. In each case the rate of increase in asparagin-nitrogen paralleled rather closely that for decrease in protein-nitrogen, yet certain differences occurred which indicated that other amino acids were being formed. In the light of the more recent work of THOMPSON (36) and ZLATAROFF (38), such a conclusion seems warranted, although they used different kinds of seeds. Comparison of PRIANISCHNIKOW's results on *L. luteus* with those of MERLIS (20) on *L. angustifolius* indicates that there was a considerable difference in the rate of protein metabolism between these closely related species. SCHULZE (32) believed that such differences might be attributed to differences in the qualitative nature of the proteins in the seed.

MILLER (22) pointed out that different seeds showed a difference in the digestion of fats similar to the difference in protein digestion just discussed. In this article may be found an extensive bibliography and review of the literature. Here especially there seems to have been a relationship between chemical properties of the original reserves, the nature and rate of change, and the products formed.

IVANOW (13) and MAQUENNE (19) attributed this to the proportion of saturated to unsaturated fats.

The qualitative and quantitative changes in the reserve food materials during the development of seedlings may be modified greatly by changes in the environmental conditions. Temperature was found by PRIANISCHNIKOW (31) to influence the rate of protein metabolism in germinating peas. Closer examination of his data indicates that the influence was more marked between eight and thirteen days than at earlier periods. WASNIEWSKY (37), working with wheat seedlings, showed that temperature changes affected the digestion of starch and protein differently. Small changes in temperature at which potatoes were stored were very effective, according to HOPKINS (11), in producing differences in the rate of sugar accumulation. FISCHER (6) demonstrated that the fats stored during the winter in the twigs of the so-called "fat trees" were rapidly converted to starch when subjected to higher temperatures, the rate of change being proportional to the temperature. When again placed at lower temperatures the reverse of this process took place.

It is evident, therefore, that external conditions alter the metabolic processes of plants. The extent to which this is possible seems to depend upon several internal factors, such as the stage of development, composition of the reserve materials, etc. The different results obtained in comparative studies on respiration may be related to such factors, and the effect of the environment on them, but this is still an open question.

The rate of respiration of *Vicia faba*, *Pisum sativum*, and *Lupinus luteus* throughout their development was studied by PRIANISCHNIKOW (30). In each of these he obtained a "grand period" curve, but the respective curves showed clearly that there were individual differences. KUIJPER (17), where will be found a comprehensive review of the literature, determined the effect of temperatures between 0° and 50° C. on the hourly rate of respiration of lupines, peas, and wheat. Differences in the respiration of these plants between certain limits of temperature were not the same as they were between other limits. POURIEWITSCH (27) studied the respiration of seedlings of five species of plants, representing four genera, at different stages in their development. Temperature influ-

enced the value of the respiratory quotient, the effect being more marked in young seedlings than in older ones. GORE (9) determined the effect of temperature on the respiration of different varieties of fruits, and found that they varied even when grown in the same orchard. The difference was more marked at high temperatures than at lower ones. POURIEWITSCH concluded that the effect of temperature on the respiratory quotient was dependent upon the qualitative nature of the nutrient materials in the tissues of the plant. KUIJPER (17) drew essentially the same conclusion. Quantitative chemical analyses were not made in either of these cases.

GERBER (8) argued from his results with flax and castor bean that the value of the respiratory quotient depended upon the "ease" with which the fats were oxidized. IVANOW (14) believed his experiments with sprouting seeds rich in oil showed that temperature exerted a considerable influence upon the coefficient of respiration. His position has been summarized as follows: "This value was said to depend upon external conditions and upon the nature of the plant and was not constant for any group (of conditions?) with oil-containing seeds." DELEANO (3) found that detached leaves of the grape respired only carbohydrates, chiefly starch, during the first 100 hours in the dark. After this there was a marked change in the respiratory process in which the proteins were especially affected. HASSELBRING and HAWKINS (10), BAILEY and GURJAR (1), and HOPKINS (11), working with sweet potatoes, wheat, and Irish potatoes respectively, concluded that the intensity of respiration was correlated with the sugar content, more especially the reducing sugar content. PALLADIN (24) attempted to establish a relation between the intensity of respiration and the content of nitrogen not digestible in gastric juice (protoplasmic nitrogen). He found a better correlation to exist in wheat than in lupine. SPOEHR and MCGEE (34), in their recent work on the respiration of detached leaves of sunflower and "Canada Wonder" bean, grown on nutrient solutions containing different sugars and amino acids, showed a relationship between the intensity of respiration, sugar content, and amino acid content. In general they found that an increase of amino acids increased the rate of respiration when the leaves contained an ample supply of carbohydrates. In the experiments which were conducted at differ-

ent temperatures, 20° and 24° C., the data seem to indicate that the change in the amino acid and sugar content may have been due to the temperature and the amount of the respective substances present at the start. PFEFFER (25) was able to demonstrate that the utilization of organic foods by fungi depended upon the quantitative and qualitative relations of these materials in the nutrient solution. He also found that temperature modified the results obtained. POURIEWITSCH (28) concluded that these relations were reflected in the value of the respiratory quotient of *Aspergillus niger*, one of the plants used by PFEFFER.

### Methods

Two varieties of soy bean, Manchu and Midwest, grown in 1924 under similar field conditions from certified seed, were obtained from the Illinois Agricultural Experiment Station and stored in tin containers in the laboratory until used. Lots of each variety containing carefully selected beans of equal weight were planted in sand of optimum moisture content (45 per cent of the holding capacity), and placed in the constant temperature cases. At the same time a similar lot of each was placed in the drying oven for moisture determination. When the beans had germinated so that the average length of the radicle was approximately 4 cm., the seed coats were removed and 25-30 selected plants from each lot were transferred to paraffined wire nets over 400 cc. of sterilized tap water. The cultures were then placed in the respiration chambers at the respective temperatures at which they were germinated, 15°, 20°, and 25° C. The respiration rate of these (in the dark) was determined in 24-hour intervals until there was evidence of starvation.

Throughout these experiments the aeration method was used. Before gas was collected for analysis, carbon dioxide-free air was drawn through the plant chamber for one hour at such rate that the volume of air was changed at least three times. After this the chamber was connected to an aspirator bottle which served both as an aspirator and as a means for collecting the gas. It contained a specially prepared liquid, furnished by Professor S. W. PARR of the Chemistry Department, which was known to absorb only the slightest trace of carbon dioxide. By means of this aspirator, specially

designed for use in these experiments,<sup>2</sup> it was possible to regulate and maintain a constant rate of aspiration at all times. The rate of aspiration was so adjusted that the concentration of carbon dioxide in the chamber did not exceed 0.5 per cent at any time.

The air drawn into the chamber was first passed through a 40 per cent solution of potassium hydroxide in Winckler tubes (3-foot) to remove the carbon dioxide. A sufficient length of glass tubing was used within the temperature case to bring the temperature of the incoming air to the temperature of the chamber. The intake tube of the chamber extended below the nutrient solution of the culture, so that this was being aerated constantly; hence carbon dioxide, which might have been absorbed by it, was removed by mass action.

The gas collected was analyzed by means of a Morehead burette, according to the method described by PARR and VANDAVEER (23). The volume was determined with a gas meter graduated to hundredths of a liter, but which allowed accurate estimates of one-thousandth part of a liter. This was reduced to 0° C. and 760 mm. mercury pressure. The results of the respiration rate determinations are expressed on the basis of milligrams of CO<sub>2</sub> emitted per hour per gram of original dry weight. In order to make this dry weight basis more absolute, the weight of the seed coats was deducted from the original dry weight of the beans. Since any measure of the stage of development of the plants would be largely arbitrary, it is here expressed as the number of hours following the planting of the seeds.

The respiration chambers used in these experiments were made from a good quality of tin and provided with tightly fitting covers. They were 360 mm. high and had an inside diameter of 160 mm. An intake tube was soldered in near the top of the chamber and an outlet tube near the bottom. Before each series of experiments these chambers were immersed in water and tested for leaks at approximately 100 mm. mercury pressure. They were then disinfected with a strong solution of formalin, rinsed with distilled water, and aerated with a stream of air which had first passed through concentrated sulphuric acid. Adequate precautions were taken throughout to prevent errors in the results due to contamination of the cultures by microorganisms.

<sup>2</sup> To be described in a forthcoming article.

It occasionally became necessary to remove some of the plants from the chamber because of injury, abnormalities of growth, or an indication of infection. Since all of the beans in each culture were of the same weight originally, it was possible to do this and by proper

TABLE I  
RATE OF RESPIRATION OF MANCHU AND MIDWEST SOY BEANS AT 25° C.

TIME AFTER PLANTING IN HOURS	CO <sub>2</sub> PER HOUR PER GM. OF ORIGINAL DRY WEIGHT (MG).					
	Manchu			Midwest		
	1	2	Average	1	2	Average
From To						
72-96.....	1.201	1.269	1.235	1.075	1.130	1.102
96-120.....	1.982	1.963	1.973	2.542	2.744	2.642
120-144.....	2.191	2.135	2.163	1.386	1.514	1.450
144-168.....	2.676	1.712	2.194	1.730	2.055	1.892
168-192.....	1.647	1.576	1.612	1.096	1.747	1.421
192-216.....	1.020	1.138	1.079	1.450	0.899	1.175
216-240.....	1.903*	1.253	(1.253)	0.883	0.975	0.929
240-264.....	1.130†	1.282	(1.282)	0.790	0.930	0.860
264-288.....	1.186	0.781	0.984	1.258	0.833	1.046
288-312.....	0.216	0.474	0.345	0.986	0.831	0.909
312-336.....		0.602	0.602	0.854	0.456	0.655
336-360.....		0.876	0.876	0.755	0.509	0.642
360-384.....		0.615	0.615	0.680	0.416	0.548
384-408.....		0.441	0.441	0.603	0.925	0.764
408-432.....		0.244	0.244	0.643	1.048	0.845
432-456.....	0.285	0.513	0.399	1.087	1.197	1.142
456-480.....	0.361	0.551	0.406	1.134	0.663	0.898
480-504.....	0.546	0.775	0.661	1.210	0.481	0.846
504-528.....	0.381	0.596	0.488		0.907	0.907
528-552.....		0.783	0.783			
552-576.....		0.748	0.748			
576-600.....		0.713	0.713			

\* Temperature of case up to 30° C. during the night.

† Temperature of case down to 20° C. for four hours.

Neither of these values considered in the average.

calculations to retain the dry weight basis. It was seldom necessary to remove more than a few, so that the space relations were not appreciably disturbed.

## Results

VARIATION IN RESPIRATION.—The results of the respiration rate determinations are found in tables I, II, and III, and are shown graphically in figs. 1, 2, and 3. It will be seen that there is great variation in the rate of respiration of either variety of soy bean

TABLE II

RATE OF RESPIRATION OF MANCHU AND MIDWEST SOY BEANS AT 20° C.

TIME AFTER PLANTING IN HOURS	CO <sub>2</sub> PER HOUR PER GM. OF ORIGINAL DRY WEIGHT (MG.)		TIME AFTER PLANTING IN HOURS	CO <sub>2</sub> PER HOUR PER GM. OF ORIGINAL DRY WEIGHT (MG.)	
	Manchu	Midwest		Manchu	Midwest
From To			From To		
96-120.....	1.218	1.137	408-432.....	1.150	0.454
120-144.....	1.435	1.465	432-456.....	0.658	0.474
144-168.....	1.984	1.840	456-480.....	1.248	0.791
168-192.....	2.426	2.046	480-504.....	0.371	0.371
192-216.....	2.199	1.278	504-528.....	0.482	0.313
216-240.....	1.385	0.865	528-552.....	0.829	0.674
240-264.....	0.883	1.410	552-576.....	0.984	0.621
264-288.....	0.939	1.147	576-600.....	0.786	0.820
288-312.....	0.962	1.057	600-624.....	0.737	0.653
312-336.....	0.724	0.480	624-648.....	0.775	0.454
336-360.....	0.856	0.480	648-672.....	.....*	0.588
360-384.....	0.414	0.687	672-696.....	.....	0.555
384-408.....	1.275	0.821			

\* Culture too badly contaminated for further use.

TABLE III

RATE OF RESPIRATION OF MANCHU AND MIDWEST SOY BEANS AT 15° C.

TIME AFTER PLANTING IN HOURS	CO <sub>2</sub> PER HOUR PER GM. OF ORIGINAL DRY WEIGHT (MG.)		TIME AFTER PLANTING IN HOURS	CO <sub>2</sub> PER HOUR PER GM. OF ORIGINAL DRY WEIGHT (MG.)	
	Manchu	Midwest		Manchu	Midwest
From To			From To		
240-264.....	.....	0.438	696-720.....	0.654	.....
264-288.....	0.786	.....	720-744.....	0.435	0.321
288-312.....	.....	0.814	744-768.....	0.195	0.271
312-336.....	0.416	.....	768-792.....	0.122	0.415
336-360.....	.....	0.782	792-816.....	0.306	0.293
360-384.....	0.248	.....	816-840.....	0.105	0.463
384-408.....	.....	1.066	840-864.....	0.321	0.483
408-432.....	0.281	.....	864-888.....	0.365	0.898
432-456.....	.....	1.174	888-912.....	0.624	1.358
456-480.....	0.265	.....	912-936.....	0.549	0.651
480-504.....	.....	0.530	936-960.....	0.396	0.224
504-528.....	0.233	.....	960-984.....	0.439	.....†
528-552.....	0.243	0.643	984-1008.....	0.547	0.174
552-576.....	0.389	.....	1008-1032.....	0.510	0.162
576-600.....	0.373	0.803	1032-1056.....	0.307	0.201
600-624.....	0.390	.....	1056-1080.....	.....‡	0.074
624-648.....	0.349	0.428	1080-1104.....	.....	0.344
648-672.....	0.275	.....	1104-1128.....	.....	0.093
672-696.....	0.577	.....*			

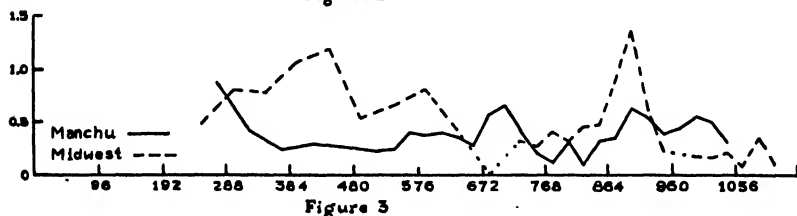
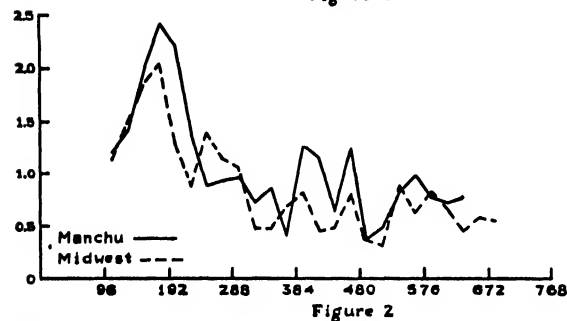
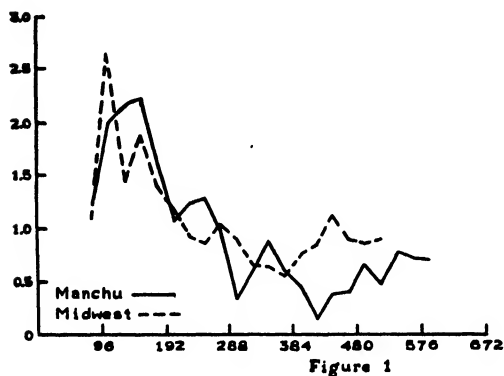
\* Value too small to measure accurately.

† Accident to burette made reading uncertain.

‡ Culture too badly contaminated for further use.



throughout its development. There is no regular periodicity in this variation, nor do the fluctuations show consistent similarity at all temperatures, even when allowance is made for the relative differ-



FIGS. 1-3.—Fig. 1, rate of respiration of Manchu and Midwest soy beans at 25° C.: abscissas represent time in hours; ordinates represent mg. CO<sub>2</sub> emitted per hour per gm. of original dry weight of seeds; values from table I; fig. 2, rate of respiration of Manchu and Midwest soy beans at 20° C.: abscissas represent time in hours; ordinates represent mg. CO<sub>2</sub> emitted per hour per gm. of original dry weight of seeds; values from table II; fig. 3, rate of respiration of Manchu and Midwest soy beans at 15° C.: abscissas represent time in hours; ordinates represent mg. CO<sub>2</sub> emitted per hour per gm. of original dry weight of seeds; values from table III.

ence in the rate of development. The greatest variation in rate is found to occur during the early periods of development (excepting that of the Manchu variety at  $15^{\circ}$  C.), and again immediately preceding the time when the majority of the plants in the culture succumb to starvation. The two varieties differ with respect to the degree and manner of variation in the rate of respiration. During one period of development that of the one variety fluctuates to a greater extent and in a different manner from that of the other. These differences are often entirely reversed at other periods, or at least greatly changed. The same is true, in general, in regard to the relative intensities of respiration.

TEMPERATURE AND RESPIRATION.—Comparison of the results obtained at different temperatures indicates that small differences in temperature may have a greater influence upon the respiration of one variety of a species than upon another. At  $25^{\circ}$  C. the intensity of respiration is, in general, higher and tends to vary less in the Midwest variety than in the Manchu. The rate of respiration of the Midwest at  $20^{\circ}$  C. fluctuates less but is, with the exception of one 72-hour period, lower than that of the Manchu. At  $15^{\circ}$  C. the Midwest variety not only shows a greater variation in its respiration rate, but a much more intensive respiration at nearly all periods during its development. Each variety has a well defined maximum during the early stages of development at  $25^{\circ}$  and  $20^{\circ}$  C.; at  $15^{\circ}$  this is found only in the Midwest variety. During the early stages of development, and again just before exhaustion, the difference in the respiration of these varieties is much greater at  $25^{\circ}$  and at  $15^{\circ}$  than at  $20^{\circ}$  C.

RESPIRATION AND DRY WEIGHT LOSS.—Since the results of the respiration rate determinations are expressed on the basis of the original dry weight of the seeds, it might be argued that the differences obtained would be less if the dry weight of the seedlings at the beginning of each determination was used as the basis. A preliminary experiment was made to test this point. A large number of plants were grown from seeds of equal weight as just described. Ten plants were removed from the cultures at equal periods of time, every 48 hours at  $25^{\circ}$ , and every 72 hours at  $20^{\circ}$  C., and dried to constant weight in the drying oven. The results of this experiment

are summarized in table IV and shown graphically in figs. 4 and 6. It is realized that the error of these results may be quite large, due to the small number of plants used, yet the data allow certain conclusions. At 25° C. the actual loss in dry weight by both varieties, during any period, is as nearly similar as is the rate of loss. At 20° C. the actual loss in dry weight for any period is greater in the Manchu than in the Midwest variety. The differences in the respira-

TABLE IV

LOSS OF DRY WEIGHT BY MANCHU AND MIDWEST SOY BEANS AT 25° AND 20° C.; VALUES EXPRESS AVERAGE OF 10 PLANTS

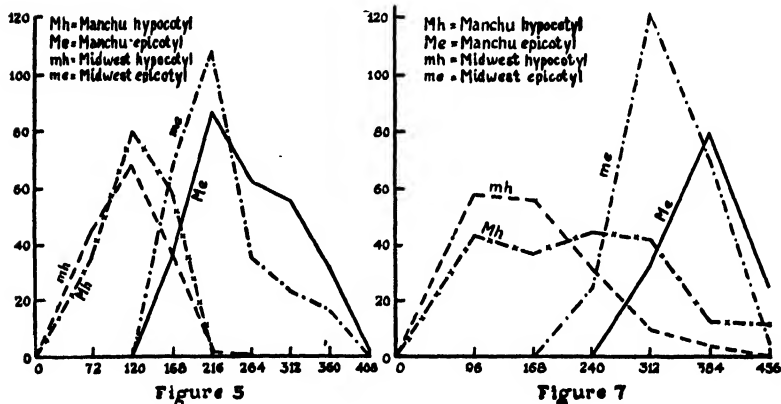
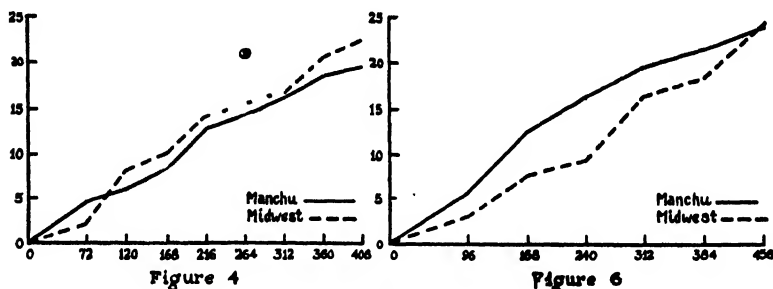
TIME AFTER PLANTING IN HOURS	AVERAGE PERCENTAGE LOSS IN DRY WEIGHT OVER ORIGINAL DRY WEIGHT OF SEEDS			
	25° C.		20° C.	
	Manchu	Midwest	Manchu	Midwest
0.....	0	0	0	0
72.....	4.48	1.85	.....	.....
96.....	.....	.....	5.75	3.45
120.....	6.08	8.21	.....	.....
168.....	9.84	10.41	12.74	7.76
216.....	12.84	14.38	.....	.....
240.....	.....	.....	16.70	9.51
264.....	14.27	(21.44)*	.....	.....
312.....	16.46	17.03	20.06	17.03
360.....	18.83	21.00	.....	.....
384.....	.....	.....	22.03	18.79
408.....	19.96	23.03	.....	.....
456.....	.....	.....	24.68	24.97

\* Value probably due to error as considerable sand adhered to roots.

tion of the two varieties at either temperature, therefore, would be magnified rather than reduced if based upon the dry weight of the seedlings at the beginning of each determination. At 20° the relative difference obtained by this method of calculation would be increased more than at 25° C., for the reasons already mentioned.

RESPIRATION AND GROWTH.—It was thought that the variation in the rate of respiration might in some way be related to the periodicity in the rate of growth, and that this might account for some of the differences in the respiration of the varieties. Accordingly, when the plants were removed from the cultures for dry weight determinations, measurements were made to determine the increment of

growth, both of the hypocotyl and epicotyl. The average increment of growth of ten plants is given in table V. These values are reduced



FIGS. 4-7.—Fig. 4, rate of loss of dry weight by Manchou and Midwest soy beans at 25° C.: abscissas represent time in hours; ordinates represent percentage loss over original dry weight of seeds; values from table IV; fig. 5, rate of growth of Manchou and Midwest soy beans at 25° C.: abscissas represent time in hours; ordinates represent increment of growth in mm.; values from table V; fig. 6, rate of loss of dry weight by Manchou and Midwest soy beans at 20° C.: abscissas represent time in hours; ordinates represent percentage loss over original dry weight of seeds; values from table IV; fig. 7, rate of growth of Manchou and Midwest soy beans at 20° C.: abscissas represent time in hours; ordinates represent increment of growth in mm.; values from table V.

to graphs in figs. 5 and 7. As already mentioned, the possibility of error is large. It will be seen, however, that the rate of growth of each part of the shoot increases to a maximum and then decreases,

yet noticeable differences seem to exist between the two varieties. These differences are more apparent at 20° than at 25° C. A direct correlation between the growth rate and respiration does not seem possible in the case at hand. Neither is it likely that the differences

TABLE V

GROWTH IN MM. OF MANCHU AND MIDWEST SOY BEANS AT 25° AND 20° C.;  
VALUES EXPRESS AVERAGE OF 10 PLANTS

TIME AFTER PLANTING IN HOURS	MANCHU				MIDWEST			
	HYPOCOTYL		EPICOTYL		HYPOCOTYL		EPICOTYL	
	Length	Growth	Length	Growth	Length	Growth	Length	Growth
Temperature 25° C.								
0.....	0	0	0	0	0	0	0	0
72.....	35	35	0	0	45	45	0	0
120.....	115	80	0	0	113	68	0	0
168.....	174	59	36	36	148	35	68	68
216.....	165	(o)*	123	87	150	2	177	109
264.....	179	(o)	186	63	143	(o)*	212	35
312.....	180	(o)	242	56	152	(o)	235	23
360.....	176	(o)	274	32	149	(o)	252	17
408.....	176	(o)	276	2	148	(o)	241	(o)*
Temperature 20° C.								
0.....	0	0	0	0	0	0	0	0
96.....	43	43	0	0	58	58	0	0
168.....	80	37	0	0	114	56	0	0
240.....	125	45	0	0	145	31	24	24
312.....	167	42	32	32	155	10	146	122
384.....	180	13	112	80	159	4	215	69
456.....	192	12	136	24	154	(o)*	220	5

\* Growth increment considered as o due to the possible error resulting from the use of small numbers

in the respiration rate of the two varieties might be explained on the basis of differences in the rate of growth.

### Discussion

Soy beans were selected for use in this investigation because they possess several advantages from an experimental point of view. The inherent nature of most autotrophic plants makes difficult the kind of experimentation employed by the animal physiologist to determine the fundamental principles of respiratory metabolism. Be-

cause of this it has been customary to employ highly artificial methods, or to use plants which were known to have different kinds and amounts of reserve food materials. Either of these methods may lead to an incorrect interpretation of the respiratory metabolism of the plant, as will be shown later. The use of soy bean varieties overcomes several of these difficulties. According to PIPER and MORSE (26), soy bean seed contains 15-25 per cent fat, 30-45 per cent protein, and no starch if the seed is properly matured. The limited number of reserve food materials in this seed is of distinct advantage in comparative studies of respiratory metabolism. Traces of carbohydrates have been found in soy bean seed, but the authors referred to consider the available data widely divergent and conflicting. Several instances are cited where various investigators claim to have found 2 to 3 per cent of sugar, traces to 4 per cent of pentosans, and other carbohydrates in small quantities. In any case the amounts are comparatively small, and might be expected in any of the seeds generally employed in investigational work. Another advantage lies in the fact that the composition of the seed of soy bean varieties varies quantitatively. This makes possible the selection of seeds of varieties which contain different amounts of the two principal reserves. The relative amounts of the respective reserves are altered by cultural conditions, as was shown by STARK (35). Under similar field conditions, one variety may be designated as having a "low oil-high protein" seed and another variety as "high oil-low protein" seed. When the same varieties are grown under dissimilar field conditions this might be reversed. The seeds used were chosen with this in view. It will be shown later that the use of the two varieties of soy bean chosen largely eliminated the possibility of the reserve materials varying qualitatively.

**VARIATION IN RESPIRATION.**—As already pointed out, there is a great variation in the rate of respiration during the development of soy beans in the dark. In general the variations are characterized by rapid increases in rate, which are followed by gradual decreases, and by the absence of regular periodicity. In this respect the results differ quite widely from those obtained by PRIANISCHNIKOW (30), and from the conclusions he draws from the work of others (RISCHAWI, MAYER, etc.). The rate of respiration in their studies showed no

marked variations. They found the rate to increase gradually to a maximum, and then to decrease gradually as the reserve materials were depleted. When variations were obtained they were always slight. The maximum respiration was found by them to occur late in the development of the seedlings. In the present study, aside from the variations mentioned, the maximum rate of respiration occurs during the first stages of development, with the exception of that of the Manchu variety at 15° C. This difference in results might be due to two things. It might be attributed to the influence of the small amount of carbohydrates present in the seeds. This is hardly possible, however, because the greater part of these carbohydrates has been shown to be pentosans. Wheat has been shown by LE CLERC and BREAZEAL (18) to contain as much as 7.3 per cent of this material. RISCHAWI used this plant in his studies, and obtained no great increase in the rate of respiration during the early stages of development. Further, all of the chemical analyses made on development of seedlings in the dark indicate that the most rapid change in the several reserve foods occurs during the early periods of development. This would obscure the effects of the small amount of carbohydrates present in soy beans. Since the differences are not likely to be due to the pentosans present, they might be attributed to the differences in the method of expressing results. In all of the early works mentioned, the rate of respiration was expressed as the milligrams of carbon dioxide given off by a certain number of plants per hour. This method of expression, unless the dry weight of the seeds is identical, allows considerable error when comparative results are desired.

The variation in the respiration rate of the soy bean varieties is more marked at some temperatures than at others. The two varieties differ from each other especially in this respect. At 25° C. both varieties show the greatest fluctuation at the beginning and again near the end of the development, but at 20° the rapid change near the end of development is lacking in the Midwest variety. At 15° there is no marked increase in the respiration of the Manchu variety during the early periods. The Midwest variety shows a rapid increase both near the beginning and again near the end of its development at this temperature. This does not seem to bear out the conclusion

of POURIEWITSCH (27) that the effect of temperature on respiration is more marked in early stages of development. GORE (9) found that the difference in the respiration of varieties of fruits was more marked at high temperatures than at lower ones. This is not the case with the soy bean varieties used in this investigation. It can be concluded, therefore, that the effect of temperature upon the respiration of plants may be as marked during the early periods of development as during the later periods. Also, that the effect of temperature on respiration is as different for varieties of the same species as it is for different species.

RESPIRATION AND COMPOSITION.—The results obtained by the methods here used would indicate that certain revisions in the current conception of the relation between respiration and chemical composition of the reserves are necessary.

It has been believed, as was shown earlier in this paper, that differences in the respiration of plants depend upon differences in the reserve food materials in the tissues. These conclusions were drawn from results obtained by the use of different species of plants, and often by the use of different genera. If conclusions are to be drawn concerning the effect of quantitative differences in the reserve materials, it seems imperative that plants be selected for experimentation in which these quantitative differences are not masked by differences of a qualitative nature. Concerning the importance of this consideration, the physiologist is at present in a position to make certain assumptions. From the work that has been done on the chemical changes accompanying the germination of fatty seeds and the development of seedlings, it is quite certain that qualitative differences in the reserve materials have a great effect upon the quantitative (and qualitative) nature of the by-products found at different stages of development. It can be assumed with some assurance that the proteins of seeds, especially of different genera of plants, are quite specific. This hypothesis is at the very foundation of the well known precipitin tests which recently have been introduced into botanical investigations by MEZ and his students (21). It is also the basis of the work of SCHULZE (32) in his investigation of the relationship between the qualitative nature of the proteins of seeds and that of the protein by-products found in the seedlings.



The assumption that the qualitative nature of the reserve materials in the seeds of the two varieties is the same has considerable basis in fact. It follows that they differ in composition only quantitatively. According to the data of STARK (35), the Midwest variety has a "low oil-high protein" seed, and the Manchu a "high oil-low protein" seed when grown under similar field conditions. Actual analysis of the seed of the varieties used in this study showed that the Midwest contained 39.37 per cent protein and the Manchu 32.19 per cent protein per unit dry weight.

The characteristics of the respiration of these varieties have been pointed out repeatedly. If differences in their respiration are to be explained on the basis of quantitative differences in the reserve food materials, several difficulties are encountered. In the first place, since the fats are the original source of the respirable materials, the fact that the intensity of respiration of the Midwest variety (low oil-high protein) is, in general, higher than the Manchu (high oil-low protein) at 25° and at 15° C. would be difficult of explanation. Secondly, KUIJPER (17) concluded that the change from one characteristic respiration rate to another was more marked at lower temperatures in those seeds having a high protein content. If this be true, the intensity of respiration should be consistently lower in the Midwest variety, which has the higher protein content. This is not the case. Moreover, the variation in the respiration of the Manchu variety is least at 15° C. (fig. 3), which does not support the conclusion mentioned. Lastly, POURIEWITSCH (27) believed that the effect of temperature upon the respiratory quotient depends upon the qualitative nature of the nutrients in the tissues of the plants. The results obtained by the writer contradict this statement. It might be argued that the measure of respiratory activity was not the same as that used by POURIEWITSCH. This is of no great consequence, however, since FERNANDES (5) has shown by very carefully performed experiments that the production of carbon dioxide and the absorption of oxygen by developing seedlings parallel each other closely. Further, the results of POURIEWITSCH must be taken with some reservation, since the checks he used showed abnormally wide variations, 0.11 and 0.54, 0.48 and 0.13, 0.35 and 0.88, for the values of the quotient.

In the discussion of the literature bearing on the changes which take place in the several reserve food materials during the development of seedlings in the dark, it was shown that the greatest change in any of these compounds occurs during the early periods of development. After this the rate of change of any one of them was continuous and gradual, or showed only slight variation. The lack of periodic changes in these materials is the chief characteristic of this early work. This was also shown to be a feature of the respiration rate determinations made on the same plants. The opinions of HASSELBRING and HAWKINS (10), BAILEY and GURJAR (1), and HOPKINS (11) were cited to show that any external conditions which caused a rapid accumulation of respirable materials also caused rapid changes in the rate of respiration. Accordingly, when the results of the respiration rate determinations are considered with respect to directly respirable materials, it appears that the changes in such materials in developing seedlings are not gradual; or, if gradual, that the plant respire different materials at different stages of its development. If either of the above premises should be proved correct, the reactive nature of the protoplasm would be emphasized, and support lent to the idea of PFEFFER (25) that there is an "election" of organic nutrients by the plant, and to the idea of POURIEWITSCH (28) that this election is reflected in respiration. PFEFFER also believed that environmental conditions, especially temperature, modified the results obtained. This is corroborated by the results obtained in this investigation.

If it be permissible to apply the work of FISCHER (6) to the results obtained with soy beans at 15° C., one of two things is evident. Either the conversion of fats to respirable materials is extremely slow, especially in the Manchu variety, or those products which are formed are not respired by the plants. This point awaits clarification, as little is known at present concerning the digestion of fats in developing seedlings at low temperatures. If the first step in the conversion of fats is the production of starch, which is not unlikely from the results of FISCHER and DETMER, consideration would have to be given to the work of HUERRE (12), who showed that the cardinal temperatures for the activity of maltase of one variety of maize were considerably lower than that for another variety. Such a possibility

strengthens the proposition that the reactive nature of the protoplasm, rather than the chemical composition as such, is at play in respiratory metabolism. In contradistinction to this, the suggestion might be derived from FISCHER's work that there is a temperature at which the formation of starch from fat, and the reverse of this process, are at equilibrium. This in no way minimizes the proposition already set forth. If it did the respiration rate of the two varieties should at least parallel each other at 15° C., which is not the case.

**RESPIRATION AND STARVATION.**—The characteristic of respiration just preceding the exhaustion of the seedlings is significant in several ways. Its prevalence at all temperatures, regardless of varietal differences, indicates that the plants act specifically when this period is reached. This has not been shown heretofore, so far as the writer is aware, or at least has not been emphasized. The supposition might be offered that certain by-products of metabolism which have accumulated are influencing the respiratory processes, either directly or indirectly, at this time. On this basis alone it would be difficult to account for differences in the respiration of the two varieties. Still more difficult would be the explanation of the inconsistencies of these differences at all temperatures. It has been demonstrated repeatedly that the intensity of respiration of all parts of the plant decreases as the tissues become older. KIDD, WEST, and BRIGGS (16) reviewed the principal literature bearing on this question, and presented evidence in support of the fact that even the meristematic tissues of plants showed a decrease in respiratory intensity as the plant increased in age. This indicates that the protoplasm of plants undergoes some change which reduces its ability to respire the reserve materials, which, under the conditions of their experiments, were increasing in amount. It therefore seems inconsistent to argue that chemical composition is alone responsible for respiratory activity. This is especially true with respect to the supposition concerning the effect of the accumulation of by-products. However, it is clear that the results of a protoplasmic change in the plants used in the present investigation lead to an increased respiratory activity. This is not in accord with the results obtained by the other investigators.

The differences in the respiration of the two varieties at the various temperatures used allows the conclusion that temperature may have as great an effect during the later periods of development as it has during the early ones. This is contrary to the conclusions of POURIEWITSCH (27).

**RESPIRATION AND GROWTH.**—Although the data presented are insufficient to allow definite conclusions, there seems to be no direct relation between respiration and growth as measured by the increase in length of the parts of the shoot; neither is such a relation evident between the periodicity of growth and that of respiration. Should changes in the intensity of respiration precede or follow changes in the growth rate, a synchronism between the curves of respiration and growth would be expected. This is not apparent in the results obtained. Considering the growth rate of the entire shoot instead of that of its parts, it can be said that the maximum rate of respiration is reached earlier in the development of the plants than is the maximum rate of growth. It will be noted, however, that the interval between the two maxima is greater at 20° than at 25° C. Consequently, the functional interactions which determine growth on the one hand and respiration on the other are affected differently by the same change in environmental conditions.

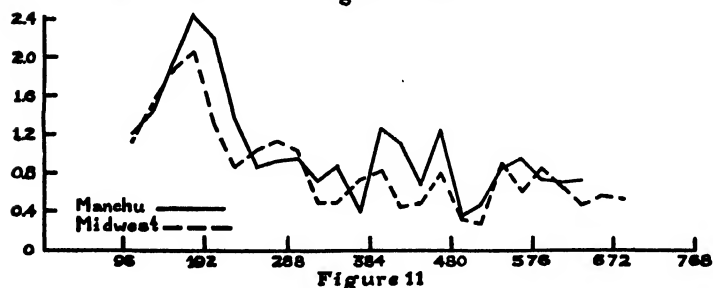
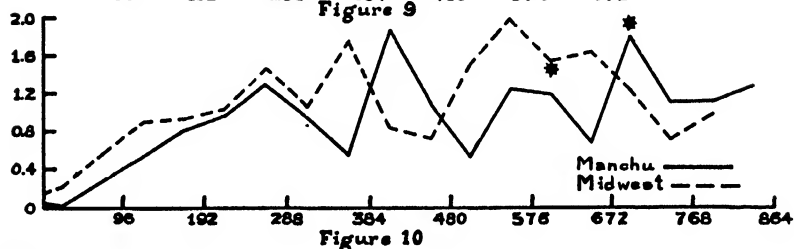
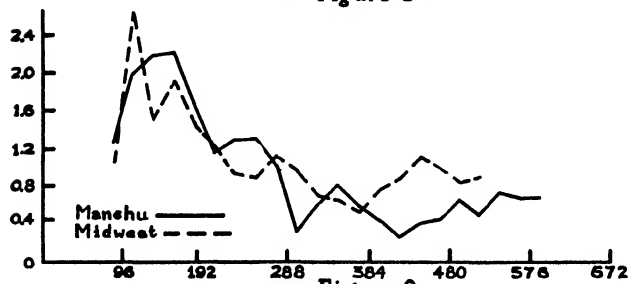
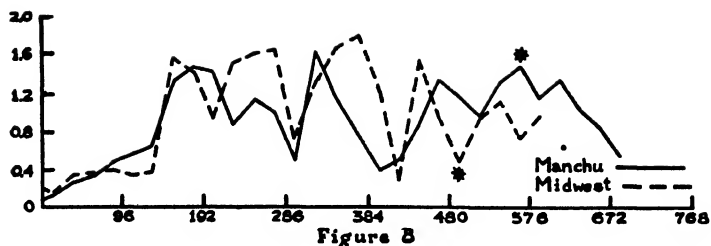
**RESPIRATION AND AMINO ACIDS.**—Thus far in this comparative study, consideration has been given only to the possible interrelationship between respiration and the reserve materials which are directly respirable. This restricted comparison would be open to criticism, since it has been shown that the by-products of the reserves which are, under ordinary conditions, not directly respirable may influence respiration. It was shown earlier in this paper (SPOEHR and MCGEE 34) that an increase in the amino acid content of excised leaves increased the rate of respiration when the leaves contained an ample supply of carbohydrates. This was especially true when the leaves were "fed" sugars and amino acids. Concerning the effect of the natural accumulation of amino acids on respiration, these writers expressed themselves as follows:

Undoubtedly the carbohydrate supply alone does not determine the rate of respiration, but there is necessary an accessory factor which aids the successive chemical reactions constituting this process. That the amino acids act

in this stimulating manner is established by the experiments here described, and the point naturally suggests itself that under normal conditions in the dark, when carbohydrate content decreases and the amino acids increase, there is a similar stimulating action which tends to maintain a relatively higher respiration rate. . . . It appears that the natural accumulation of amino acids is just as effective in stimulating respiratory activity as when amino acids are fed to the leaves.

From this it would seem that the consideration of these substances is paramount to any study of respiratory metabolism. Fortunately such a study was being carried on simultaneously in the laboratory of plant physiology at the University of Illinois. The plants used for this study were grown from the same seed as those used in the respiration studies, and under the same controlled conditions. In figs. 8 and 10 will be found the results of the amino acid determinations, made by the Van Slyke method, kindly furnished the writer by Dr. ORTON K. STARK from unpublished data. In order clearly to show the relationship between amino acids and respiration, the results obtained in the respiration studies are again plotted in figs. 9 and 11. It is necessary to point out that the determinations of the amino acids were carried out further than was possible in the respiration studies. For this reason, the time at which the cultures used in the amino acid determinations showed the same degree of exhaustion as was evident when it was necessary to terminate the respiration studies is indicated on the amino acid graphs.

Inspection of the graphs for the amino acid content shows that there is as great a variation in the amino acid content as there is in the rate of respiration. The lack of regular periodicity is equally evident, as is the absence of similarity between the curves of the two varieties. A comparison of the curve for the amino acid content of either variety with that for respiration, shows that in general there is no direct relation between amino acid content and respiration. This is especially apparent during the early stages of development, 72 to 120 hours at 25°, and 96 to 240 hours at 20° C. It will be seen that during the later periods an increase in the rate of respiration is accompanied by a decrease in the amino acid content, and vice versa. There are a few exceptions to this generalization at both temperatures, but a closer inspection of the graphs reveals the



FIGS. 8-11.—Fig. 8, amino acid content of Manchu and Midwest soy beans at 25° C.: abscissas represent time in hours; ordinates represent percentage of original dry weight which is amino acids; values from unpublished data at University of Illinois; asterisk indicates time when amino acid cultures reached stage of development necessary to discontinue respiration studies; fig. 9, rate of respiration of Manchu and Midwest soy beans at 25° C.: abscissas represent time in hours; ordinates represent mg. CO<sub>2</sub> emitted per hour per gm. of original dry weight of seeds; values from table I; fig. 10, amino acid content of Manchu and Midwest soy beans at 20° C.: abscissas represent time in hours; ordinates represent percentage of original dry weight which is amino acids; values from unpublished data at University of Illinois; asterisk indicates time when amino acid cultures reached stage of development necessary to discontinue respiration studies; fig. 11, rate of respiration of Manchu and Midwest soy beans at 20° C.: abscissas represent time in hours; ordinates represent mg. CO<sub>2</sub> emitted per hour per gm. of original dry weight of seeds; values taken from table II.

fact that when, for example, an increase in respiration is accompanied by an increase in amino acid content, the rates of the respective increases vary widely from one another. For this reason these exceptions are not regarded as significant. When the graphs expressing the rate of respiration of the two varieties are compared with those of the amino acid content, it is seen that the differences in the respiration are concomitant with differences in the amino acid content. It is significant, however, that when the rate of respiration of the one variety is higher or lower than that of the other, its amino acid content is respectively lower or higher. The slight exceptions to this, especially at 25° C., may be disposed of in the same manner as those just referred to. At 20°, where in a few instances the exceptions are more marked, no definite conclusions can be drawn concerning them. The analyses of the amino acid content were made only at 48-hour intervals at this temperature. The exceptions mentioned might be attributed to this, since the rate of respiration shows a considerable change in 24 hours at 20° C.

From these results it can be concluded that the normal accumulation of amino acids in plants grown in the dark is incidental, rather than necessary to respiration. At the best it is highly improbable that their accumulation is in any way essential to the economy of the plant in maintaining its respiratory intensity.

It could be argued, from the results of SPOEHR and MCGEE, that the decreased supply of carbohydrates in the seedlings, especially during the later periods of development, obscured the stimulating effect of the amino acids. On this basis it would be difficult to account for the fact that, during the early periods of development, the relative intensity of the respiration is in inverse relation to the relative amino acid content. During this period it is reasonable to expect, from chemical analyses which have been made by others, that the supply of carbohydrates is "ample."

The results of the respiration studies reported in this paper and those of amino acid content made on the same plants are significant in another way. The variation in the rate of respiration is rapid, great changes occurring in 24 hours. Equally great are the changes in the amino acid content during a similar period. In the discussion of the literature relative to the changes which took place in the

various reserve materials during the development of seedlings, it was shown that the changes in the several reserves were progressive and comparatively gradual. Thus it was thought that the protein content decreased gradually and the amino acids increased similarly. The same general conclusion was drawn by the various writers concerning the other reserves. Analyses were made, in the majority of these cases, at intervals of several days. From the study of respiration and amino acids reported in this paper, it is reasonable to conclude that the changes in any of the reserve foods are not gradual when the interval of determination is sufficiently short, and that a correct interpretation of this phase of metabolism requires further study.

### Summary

1. The two varieties of soy bean used in this investigation were selected in such a manner that the seeds varied in composition only quantitatively.

2. It is shown by comparative study of the two varieties that:  
(1) the rate of respiration, at all temperatures, varies greatly during the development of the seedlings without indication of periodicity;  
(2) there is a great difference between the two varieties with respect to the intensity and the fluctuation of the rate of respiration; and  
(3) the maximum rate of respiration occurs early in the development of the seedlings, with the exception of that of the Manchu variety at 15° C.

3. Temperature has a great influence upon the degree and relative rate of variation in respiration, but the effect is not the same at different stages of development, nor is it the same for each variety.

4. The effect of temperature on respiration is as markedly different in the two varieties as was that reported by others for different species of plants.

5. The conclusion of others, that differences in the respiration of different plants can be explained on the basis of quantitative differences in the reserve food materials, is not supported by the results obtained in this investigation.

6. Evidence is presented which supports the theory of PFEFFER that plants show an "election" of organic nutrients, and the results of POURIEWITSCH that this election is reflected in respiration.



7. Soy beans grown in the dark show a marked increase in the rate of respiration preceding the complete exhaustion of the plants. This has not been demonstrated previously.

8. No direct relation between respiration and growth seems to exist from the data obtained in this investigation.

9. The accumulation of amino acids in soy bean seedlings grown in the dark is incidental, rather than necessary to respiration. No evidence was obtained, under the conditions of these experiments, to support the theory that the amino acids stimulate respiration.

10. Evidence is presented to show that the changes in the reserve food materials in developing seedlings is not progressive and gradual, as heretofore has been supposed.

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## REVISION OF THE GENUS BEJARIA MUTIS

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### Historical sketch

The first specimen of the genus *Bejaria* was found by MUTIS in 1761 in Colombia (New Granada), near Santa Fé de Bogota, on calcareous declivities (2800–3200 m.). MUTIS sent the specimen to LINNAEUS, and called it *Bejaria* as a compliment to his friend BEJAR, a professor of Cadiz. LINNAEUS, misreading *j* for *f*, published it under the erroneous name of *Befaria aestuans* in *Mantissa* 1771, p. 242, where it was faultily mentioned as native of Mexico. A little later MUTIS sent another specimen from the same place, and in LINNAEUS' *Supplementum* we find two species of *Befaria*, *B. aestuans* and *B. resinosa*, mentioned thus early as coming from Colombia. During a long time the new genus was quite unknown in Europe, and no herbarium possessed a specimen of it. There had reached Sweden nothing but a single description, and that of *B. aestuans*. That is the reason why RUIZ and PAVON, the Peruvian explorers, having found some representatives of *Bejaria* in Peru, described them as a new genus, *Acunna*, with two species, *A. oblonga* and *A. lanceolata*. The identity of *Acunna* with the genus *Bejaria*, however, was established beyond question by ZEA, pupil of MUTIS, and later by VENTENAT (*Syst. Veg. flora Peruv.*).

The unwarranted segregation of the genus *Acunna* having crept into literature, many authors have repeated it until the present day; this notwithstanding the fact that ZEA, CAVANILLE, and others had very promptly shown the error of RUIZ and PAVON. We prefer to use the real name, *Bejaria*, given by the first collector. This name might well have been spelled "*Beharia*," however, to accord with the Spanish pronunciation.

In 1800 VENTENAT (in Hort. Cels. N. et tab. 51) described a new species, *B. racemosa*, native of Florida and collected by MICHAUX. The collector intended to call it *B. paniculata*, because many of his specimens had a paniculate inflorescence; but since VENTENAT, indi-

cating such type of inflorescence as an abnormal deviation, published it under the name of *B. racemosa*, the later name of *B. paniculata* (Michx. Bor. Amer. 1803) must be taken as a synonym.

In 1809 the authors of *Nova Genera et Species*, HUMBOLDT and BONPLAND, published the description of four species brought all at one time from Colombia, Ecuador, and Peru. Then in 1837 HARTWEG found the first representatives in Mexico, *B. mexicana*, *B. laevis*, and *B. discolor*. This, however, did not prevent TURCZANINOW from describing a new genus, *Jurgensenia*, with one species, *J. mexicana*, which undoubtedly is *B. discolor*.

All these species of *Bejaria* are attractive shrubs or trees, often reaching the height of 8-10 m., with large scarlet or white flowers. *Bejaria* is called by horticulturists "the rose of the Andes," in allusion to its resemblance to "the rose of the Alps" (*Rhododendron*), a genus lacking in South America. The first attempts toward its cultivation were made late in the eighteenth century. These attempts evidently were highly successful, as is evidenced by the large lists of cultivated *Bejarias* given as early as 1850 in PAXTON and LINDLEY'S *Flower Garden*. We may add that their cultural handling is not especially difficult, although a damp atmosphere and free circulation of air in summer are no doubt essential.

### Geographical distribution

The "rose of the Andes" is chiefly distributed in the Andes of South America, reaching the southern limit of 22° S.L. in the mountain region of Bolivia. Separate representatives are found also in North and Central America, however, one species in Cuba (Pinar del Rio), four in Mexico, and one in Florida and southeastern Georgia. The northern limit of the genus is found at 30° N.L. In South America the area of *Bejaria* is coextensive with the ridge of the Andes from Venezuela to Bolivia.<sup>1</sup> Three species only are found in British Guiana, at Mount Roraima. We cannot for a certainty deny the existence of one or more representatives of the genus in Guiana between the Andes of Venezuela and Mount Roraima, be-

<sup>1</sup> In *Index Kewensis* one species, *B. sprucei*, is erroneously mentioned as native of Brazil. Tarapoto, where *B. sprucei* is found, is on the eastern extension of the Andes of Peru, and *B. sprucei* is nowhere to be found in Brazil.

cause only a few investigators have visited this country; but we may mention here that there are a number of genera of the northern Andes (as regards South America) which have passed along the coastal Andes to Silla de Caracas, and yet do not extend southward over Bolivia; also which have reached Mount Roraima, but not southern Brazil.

The genus *Bejaria* is a typical representative of the alpine and sub-alpine vegetation of the Cordilleras. It frequently reaches considerable altitudes; thus for instance, in Bolivia *B. denticulata* is found at the upper limit of vegetation. The greatest number of South American species of *Bejaria* are distributed in Colombia, where eighteen of thirty-five species were found. Many of them are endemic. There is also a great diversity of forms and conditions of vegetation in this country. The most common range of distribution is the zone where the temperature is frequently  $12^{\circ}$ – $14^{\circ}$  C. during the day and  $4^{\circ}$ – $6^{\circ}$  C. at night. SIEVERS mentions a particular region in the Sierra Nevada in Colombia, at about 2800–3100 m., where *Bejaria* is found in great quantity, giving the character to the landscape. Here it appears as a small tree 3–4 m. high, growing alone or forming little groves. In the province of Socorra and Antioquia, certain species descend to 1500 m., while at the same time *B. aestuans* and *B. phyllireaeifolia* are frequently met at the height of about 4000 m. From Colombia the species of *Bejaria* are penetrating into the Andes of Venezuela, where they are found in the dry woods of Sierra Merida and Silla de Caracas, often at the upper limit of trees. *B. glauca* was collected by FUNCK in the environs of Caracas at 1500 m., where it was growing in dry sunny and sandy places, together with *Gaultheria coccinea* and *G. odorata*; they are the most typical plants of the subalpine zone. In the woods in deep shade it often reaches 10 m. in height. The limit of its distribution is at 2000 m., where it gives place to another species, *B. ledifolia*, and often *B. grandiflora*, which here represent the alpine zone of vegetation with *Gaylussacia buxifolia*, some *Vaccinium* sp., and others. On the contrary, *B. ledifolia* grows only at an altitude of 1900 m. and upward. On the southern declivities of Silla de Caracas there are many thick groves formed by *B. ledifolia*. We have mentioned already that in the eastern part of Venezuela *Bejaria* is absent and

appears only in British Guiana, with three species on Mount Roraima at 2400 m. These species are here endemic, and represent a closely allied group. To the southwest of Colombia the genus is distributed in Ecuador, Peru, and Bolivia. In Ecuador five species are present in the Valley of Loja, in Paramo de Saraguru, at about 2000 m. Here there is a warmer climate with average temperature of about 15°–18° C. *Bejaria* grows also in Quito where the climate is more dry and cold. Two of the Ecuadorean species are endemic; the other three occur also in Colombia and Venezuela. Out of eight species growing in Peru, three only are common in Colombia and Ecuador; the other five are endemic on the eastern declivities of the west Cordillera, in the mountain basin of Caxamarca at 2700 m., in the middle Cordillera, Chachapoyas, Huanaco, Tarapoto. The climate here is drier and colder. Finally, in Bolivia "the rose of the Andes" reaches its southern limit. Here already *B. glauca* is met at the same altitude of 1500–2000 m., between the shrubs of Tres Cruces and Yungas; *B. denticulata*, a second Colombian species, is found in the alpine zone of Mount Viscahal. There are three species endemic in Bolivia, dwelling in Yungas and upon the eastern declivities of the Cordilleras. The Colombian-Venezuelan Andes, however, is the center of *Bejaria* development in the present day, and from there it is penetrating into the neighboring mountain regions of South America.

In Central America the distribution of *Bejaria* is of such character as to suggest merely a survival from the past. The species found here are more ancient and but little differentiated. Four of them are found in Mexico: in the province of Oaxaca, near Villa Tanatze, in the middle Mexican province of Zacatecas, Balanos, and at Sierra Madre in the province of Durango. All of these habitats are in the mountain region of Mexico, among the southern Cordilleras. In the central part between Colombia and Oaxaca species of *Bejaria* are absent. In the Antilles one species is found in the western part of Cuba (Pinar del Rio). Probably it originates from Central America, whence it came in the Tertiary time when there was a land bridge between Central America and the West Indian islands. A permanent isolation and differentiation brought about subsequent endemism of this species. .

In North America one species<sup>2</sup> is distributed in Florida and South Georgia. It is *B. racemosa* Vent, a relict and primitive type, a low shrub, with green, large leaf-blades and long, large racemes or panicles much exceeding the leaves. It is much more like *Rhododendron canadensis* than a species of *Bejaria* of South America. *B. racemosa* is found growing in dry pine barrens throughout the Florida peninsula to southeastern Georgia.<sup>3</sup> The most northern situation is on Cumberland Island, where it is a shrub 1.5–2 m. high, growing in dry sandy soils. In gardens around Charleston (South Carolina), where it frequently is introduced, it never flourishes.

It already has been mentioned that the name of “the rose of the Andes” was given to *Bejaria* because of its external similarity to *Rhododendron*. As a matter of fact, its large bright flowers, disposed in a dense inflorescence and often with a pattern in the throat, also its coriaceous, sometimes large, leaves call to mind many species of *Rhododendron*. In the following revision of relationships we shall have occasion to speak about a close alliance between the two so-called “roses” of Ericaceae; here, however, we shall allude only to the resemblance in their geographical distributions. *Rhododendron* is distributed in North America, chiefly in northern, northeastern, and eastern parts of the continent. Many species are frequent in the Rocky Mountains, reaching southward to the boundary of Mexico, and passing over into California. Some species are found also in Florida and Georgia, but in Mexico and farther on not one species is found. Here the “rose of the Andes” appears and takes the place of the alpine one.

### Origin and relationships

All that has been said thus far about the geographical distribution of *Bejaria* shows that the ancient distributional area was much broader than it is at the present time. The genus was distributed

<sup>2</sup> A second North American species was described by GANDOGER in Bull. Soc. Bot. France. 65:IV série. 1918; it is somewhat doubtful, however.

<sup>3</sup> Central peninsular Florida is a lake region. There are five well marked areas, the flora of each being different: high pine land, low pine land, shrub, bay heads, and hammocks. *Bejaria racemosa*, together with *Podostigma pedicellata*, *Rhexia ciliosa*, *R. serrulata*, *Aristida stricta*, and *Andropogon floridanus*, is peculiar to the formation of low pine land, where *Pinus serotina* and *P. caribaea* are the prevailing trees.



throughout North America. Probably *B. racemosa* occurred in the Appalachian mountains; originally developed as a mountain form, it spread during the glacial period down to the coastal plain. After the ice sheet had moved off the plateau, which suffered glaciation, many of the old plateau forms returned, but *B. racemosa* did not; it remained in the plain and migrated southward, where it found favorable conditions, while in other places which marked its distribution in the great Miocene forest it had become extinct because of unfavorable conditions. In Florida we find a veritable colony of northern plants. All the territory above the great terminal moraine was a country influenced by the glacial cold, where tundra conditions prevailed. All the country south of it, protected by the Alleghany Mountains, was covered by a forest composed of those species that had existed in this region and also in the far north prior to the advent of the last glacial epoch. Here is to be found a mesophytic association of plants that is or was abundant farther north. Among the plants of this association are *Bejaria racemosa*, *Elliottia racemosa*, and some *Rhododendron* species. We have thus traced the development of *Bejaria* in eastern North America during the glacial period, but previous to it the *Bejaria* species were distributed in Mexico and South America.

Notwithstanding the fact that the main proportion of species is found in South America, while only a few occur in North and Central America, we cannot indicate the Colombian-Venezuelan Andes as the geographic center of origin of *Bejaria* in early times. This genus originated undoubtedly from North America, if we may adopt a hypothesis formulated in view of the relationship of *Bejaria* and North American Ericaceae. As to its taxonomic position among the Ericaceae, *Bejaria* is here placed among the Rhododendroideae-Ledeae, with other Ericaceae having distinct petals. The group here mentioned, *Bejaria* excepted, embraces four genera, one of which is a native of Japan, two of North America, while one is widely distributed in North America and in Europe. Among the genera of the group three have an exceptional number of perianth lobes: *Elliottia* has four petals and eight stamens, *Tripetaleia* three petals and six stamens, and *Bejaria* 6-8 petals and 12-16 stamens. Only two genera have five petals and ten stamens, the number usual for a

great proportion of Ericaceae. These are (1) *Ledum*, a widely distributed circumpolar genus with five species, occurring throughout the northern and western part of North America (Rocky Mountains) and spreading down to California, and (2) a monotypic genus *Cladothamnus*, growing only in the western states from Alaska to Oregon.

Aside from these near relatives allied to *Bejaria* in the distinct petals and the form of the embryo, there is manifest a close alliance with the group Rhododendroideae, or more particularly with the Rhododendreae and their chief representative, the polymorphic genus *Rhododendron*. The reference of *Rhododendron* together with the other genera *Menziesia* and *Tsusiophyllum* to the group Rhododendreae is based by ENGLER on the petals, which are slightly united, and on a cylindric form to the embryo. But some species of *Rhododendron*, for instance *R. (Rhodora) canadensis*, have a short corolla tube and the petals nearly distinct. The form of the seeds is very similar to that in *Bejaria* and closely allied genera. But a greater resemblance exists between the two groups, namely, the uniting of the pollen with long threads into a pollen mass. We succeeded in establishing that in the group of Ledae all the genera, *Ledum* excepted, have such a pollen mass, which, according to ENGLER, is usual for Rhododendreae. On the other hand, *Menziesia*, belonging to Rhododendreae, has the pollen without threads.

The nearest relatives of our genus, however, which have originated in one genetic branch of Ericaceae coming from Asia to eastern and southern America, are evidently two genera. One is *Elliottia*, in the group Ledae, distributed in the southeastern part of the continent to the northern limit of *B. racemosa* (eastern Georgia and adjacent South Carolina). This monotypic genus is closely allied to *Bejaria*, and is very similar to *B. racemosa* in the matter of stature, in the long and broad racemose or paniculate inflorescence, in the leaves with a terminal gland on the midrib, and in a broad short calyx. The other is *Rhododendron* in the group Rhododendreae, the affinity of which was already mentioned. Probably very near also is *Ledothamnus*, placed by ENGLER in the neighboring group Rhododendroideae-Phyllodoceae. This genus is monotypic and endemic in British Guiana. The number of petals, quite distinct, is not con-

stant, but averages 5 or 6, the stamens being always twice the number of the petals. We have no specimens of this genus in the herbaria of Russia and cannot decide finally for *Ledothamnus* its degree of affinity. ENGLER distinguishes the group Phyllodoceae from Rhododendreae by the form of the seeds, these not at all winged but thick and triangulate, and by the absence of union among the pollen grains. We could not decide whether this is right for *Ledothamnus*, but for the closely related genus *Leiophyllum* it is quite exact.

Thus we see that all the relatives of *Bejaria* are distributed in North America and partly in northern Asia, and it seems to be clear that the genus originates not from South America but from North America.

Unfortunately, in the realm of phytopaleontology we have today no idea of the recent history of the genus. From the group Rhododendroideae the genus *Dendrium* (segregated from *Ledum*) is found together with *Leucothoe* and *Gaylussacia* in the Pleistocene of North America (New Jersey). In the lower Tertiary some leaves are found and described under the name *Tripetaleia almqvistii*, but not a single fragment of *Bejaria* or of closely allied genera have been found in North America.

During the Tertiary, Florida and the portion of North America which now comprises the Atlantic and Gulf Coast plains were beneath the Atlantic Ocean. The ancestors of *Bejaria* and of the allied genera *Elliottia* and *Rhododendron* were growing at that time throughout North America and probably northern Asia, a land connection being present during that time between the two continents. The North American Tertiary flora covered the entire land areas of the continent. The climate of the Eocene and Miocene periods was milder than at present, and over much of North America was somewhat subtropical in character. Also, the flora of these periods was fairly uniform in character from the Atlantic to the Pacific oceans, and from the American Mediterranean northward and into the arctic regions, and this flora, especially in the southern part of the Tertiary American continent, was of subtropical character; that is to say, in the great Tertiary forests the ancestor of our genus was growing. It was a mesophytic shrub with large evergreen leaves (bright on both sides), with a long and lax racemose (or paniculate) inflo-

rescence which exceeded the leaves and was covered with fine tomentum; flowers with five petals quite separate, as also the sepals; stamens twice the number of the petals and also free. The uniformity of flora and of climatic conditions did not contribute to differentiation, and traces of the mesophytic ancestor we find at present in *B. racemosa*, a survivor from the great Tertiary forest. The migration of *Bejaria* into Mexico began in a former epoch, in the Eocene, or Paleocene, when many northern plants reached Central America. When the representatives of *Bejaria* made their appearance for the first time in Mexico they met there very different conditions and also a foreign flora. During Tertiary times the climate of Mexico was much drier than that of North America. The differentiations of the immigrants from the north began in response to desert conditions, because the desert type of vegetation here was replacing the forest of North America. At the present time four species are growing here, belonging to two genetic branches. They are direct descendants of the ancestral type; the leaves are distinctly different and there are considerable differences in size, habit, inflorescence, and pubescence, characters partly modified by the environment. Already we see here the species differentiated into two groups; in the first group the pubescence of the branches, peduncles, pedicels, and leaf blades is deciduous or absent, while in the second group it is persistent and becoming glandulous. From Mexico the species of *Bejaria* penetrated into the Antilles, probably in the Eocene, when a land bridge connected Mexico and the West Indian islands. At the end of the Eocene and during the Oligocene and Miocene, this connection was severed. With the depression of the islands in subsequent periods, the West Indian land mass was broken up into physiographically distinct regions, and the present differentiation of the flora began at that time. This is the cause of a large number of endemic types in the West Indian flora. The unique species of our genus, *B. cubensis*, growing in Cuba, is an example of such an isolated and therefore very much changed form.

In late Miocene a land connection established itself between the two American continents, and the Tertiary flora of North and Central America penetrated to the southern continent. At this very time the formation of the Cordilleras was completed, and *Bejaria*

together with other tropical plants became distributed in the mountain region of the western Andes. Evidently here the most favorable climatic and edaphic conditions were found. This is shown by the development of *Bejaria* species; in South America there are growing thirty-six out of the forty-two species of the genus. The South American species can be regarded as direct descendants of those of Central America which have been evolved in response to mountain conditions. The diminution of the leaf blades and inflorescence (racemes transforming into corymbs or umbelliform clusters) is clearly an adaptation to the habitat.

The relationships among these species cannot be taken up in detail in a final way, because of the small number of specimens in Russian herbaria. However, it seems desirable here to sketch the lines of evolutionary development, at least so far as our material, containing many authentic specimens, will permit. It does not follow that the results represent more than the probable relationships. It is believed, however, that this attempt to place the species into natural evolutionary groups will be of service.

These species of *Bejaria* are very constant in the structure and development of the flowers and fruits, although the best criteria for the recognition of sections are wanting. The differences in the type of inflorescence and pubescence, however, are of such importance that they may be used as a basis for the sections of the genus. Thus the presence or lack of glandular hairs, the persistence or the complete disappearance of the peduncles and pedicels, and the disposition of the flowers in racemes, corymbs, or panicles are recognized as sectional characters.

The present species of *Bejaria* can be arranged in four sections; their distinguishing characteristics and their probable relationships are indicated in key form as follows: .

1. Inflorescence always a long, lax, terminal, solitary panicle or raceme, much exceeding the leaves; leaves bright green on both sides.....I. Racemosae
2. Inflorescence umbelliform or a shortened raceme, panicle or corymb with congested flowers, rarely solitary and as a rule scarcely exceeding the leaves; leaf blades mostly glaucous beneath
  - A. Peduncles and pedicels glabrous or covered with fine deciduous pubescence.....II. Laeves

B. Peduncles and pedicels covered with persistent tomentum and spreading glandular hairs. . . . . III. Glandulosae

C. Peduncles and pedicels covered with persistent tomentum and often spreading but not glandular hairs

IV. Eglandulosae

a. Inflorescence corymbose. . . . . § *Corymbosae*

b. Inflorescence racemose or paniced. . . . . §§ *Racemosae*

This arrangement is based on the assumption that the genus *Bejaria* was derived from an ancestor resembling *Elliottia* and *Rhododendron*, in which a long, racemose inflorescence and a sparse and not glandular pubescence were present. Probably the leaf blades also were pubescent, because certain species yet exhibit a strong tendency to retain the tomentum, while others have an equally strong tendency to drop it.

It has been shown that the contact of *Bejaria* with the genus most closely related to it, *Elliottia*, apparently is best represented by *B. racemosa*. This does not necessitate the assumption that the other sections have arisen through this one. Such a hypothesis would lead to the conclusion that the character of paniced inflorescence and that of the spreading pubescence of the peduncles were once developed and then lost in some groups of species. While this is possible, it seems improbable. More logical is the assumption that the *Bejaria* stalk was developed from an ancestor close to *Elliottia*. The development first concerned itself with the modification in the number of the parts of the perianth and the valves of the fruit, also in quantity of seeds. It was perhaps from such a group that *B. racemosa* was derived, probably together with some other species which vanished in course of time.

The hypothetical group already mentioned gave rise somewhat farther up to the *Laeves*. It seems true that this section is more primitive than either of the two remaining, in view of the fact that each of them exhibits some characters not possessed by *Laeves*, and indicative in each case of a higher order of development. We see here the abbreviation of the peduncle which is typical also in the succeeding groups, but in *Laeves* it is followed mostly by a protraction of pedicels and the complete disappearance of the tomentum. Leaf blades are very broad in *Laeves* (or, if narrow, not so thick and coriaceous in texture) and quite glabrous, but sometimes glaucous beneath.

In order to reach the remaining two groups it is necessary to leave the line which culminates in the most highly modified species of *Laeves*, and pick up a new evolutionary thread somewhere apart from the basis of *Laeves*. This is necessitated by the appearance of a very important character, namely the dense and persistent tomentum of the peduncle and pedicels. This tomentum is often followed with spreading hairs, glandular in the *Glandulosae*. The sections *Glandulosae* and *Eglandulosae* are closely allied, and this is a case of parallel variation in two groups of species not widely separated phylogenetically. The species of the *Eglandulosae* may be divided into two groups because of two different types of inflorescence, *Racemosae* and *Corymbosae*, the first with the racemose, the second with the corymbose, type. Both groups are so much alike that there can be no doubt as to their close phylogenetic origin. In the *Corymbosae*, however, the abbreviation of the inflorescence is carried to a high type, and is followed by the enlargement of the flower and other characters of high specialization not reached by the *Racemosae*.

### Key to species of *Bejaria*

- I. RACEMOSAE.—Flowers disposed in a solitary raceme or panicle much exceeding the large green leaves; one species in North America.....1. *B. racemosa*
- II. LAEVES.—Flowers disposed mostly in a corymb or shortened raceme; pedicels glabrous
  1. Flower stalks protracted, much longer than the flowers; peduncle short, inflorescence large
    - a. Leaf blades broad, ovate or elliptic, obtuse
      1. Branches covered with spreading hairs... 2. *B. subsessilis*
      2. Branches glabrous..... 3. *B. drymifolia*
    - b. Leaf blades narrow, lanceolate
      1. Leaf blades green beneath..... 4. *B. laevis*
      2. Leaf blades glaucous beneath..... 5. *B. ghiesbreghtiana*
  2. Flower stalks not protracted, scarcely or no longer than flowers, peduncle very short; inflorescence narrow, corymbose
    6. *B. glauca*
- III. GLANDULOSAE.—Flowers disposed in 1-5 racemes, panicles, or corymbis; peduncle and pedicels covered with glandular hairs

1. Flowers in terminal, solitary, lax racemes; leaves nearly linear, strongly revolute; Cuba.....7. *B. cubensis*
2. Flowers in terminal, sometimes axillary racemes or panicles with a short peduncle
  - a. Glandular hairs deciduous, flower stalks becoming glabrous; leaf blades broad, 3.5–6 cm.; inflorescence many-flowered, broad.....8. *B. discolor*
  - b. Glandular hairs persistent, but the gland-top deciduous; flower stalks always pubescent: leaf blades small, 1.5–2.5 cm.; inflorescence a few-flowered corymb.....9. *B. mexicana*
  - c. Glandular hairs persistent
    1. Inflorescence large, paniced, or the flowers disposed in 1–5 racemes; leaf blades always pubescent beneath
      - a. Inflorescence terminal and axillary; leaf blades dark beneath.....10. *B. sprucei*
      - b. Inflorescence terminal; leaf blades glaucous beneath (except in *B. denticulata*)
 

Leaf blades glandular-pubescent, green or scarcely glaucous beneath

Leaf blades minutely toothed, branches covered with black pubescence.....11. *B. denticulata*

Leaf blades entire, branches covered with ferruginous pubescence.....12. *B. guianensis*

Leaf blades tomentose

Leaf blades pubescent beneath only; petals seven

13. *B. antioquiæ*

Leaf blades pubescent on both sides; petals six

14. *B. hispida*
    2. Flowers disposed in racemes much exceeding the leaves; leaf blades very pubescent on both sides when young, then glabrous except as to glandular hairs of midrib beneath
 

Petals six; calyx lobes united about the middle

15. *B. lindeniana*

Petals seven; calyx lobes distinct nearly to base

16. *B. aestuans*
3. Flowers disposed in corymbose or umbellate inflorescence,



this scarcely exceeding the leaves; leaf blades glabrous, small, with glandular hairs on midrib

Leaf blades almost flat, with slightly revolute margin

Petals seven; flowers pink. . . . . 17. *B. densa*

Petals 6-8; flowers pink or purple

Calyx and corolla glabrous. . . . . 18. *B. imthurnii*

Calyx and corolla viscid. . . . . 19. *B. resinosa*

Leaf blades very revolute, with glandular hairs

20. *B. ledifolia*

IV. EGLANDULOSAE.—Peduncle and pedicels tomentose, without glandular hairs

*Racemosae*.—Flowers disposed in racemes or panicles

1. Leaf blades pubescent, pubescence persistent; flower stalks much longer than calyx

a. Peduncles tomentose

1. Branches glabrous; leaf blades acute; petals lanceolate

21. *B. pallens*

2. Branches pubescent; leaf blades obtuse; petals obovate

22. *B. mathewsii*

b. Peduncles covered with spreading hairs

1. Flowers in racemes, leaf blades minutely toothed

23. *B. subserrata*

2. Flowers in panicles, leaf blades entire. . . 24. *B. boliviensis*

2. Leaf blades pubescent when young, then mostly glabrous; flower stalks twice length of calyx. . . . . 25. *B. grandiflora*

3. Leaf blades always glabrous; flower stalks 3-5 times length of calyx

a. Leaf blades broad, obtuse; flower stalk reaching scarcely 1 cm. . . . . 26. *B. parviflora*

b. Leaf blades narrow, acute; flowers longer than 2 cm.

27. *B. decora*

*Corymbosae*.—Flowers in corymbs

1. Leaf blades pubescent, leaves with revolute margins

a. Leaf blades covered with spreading hairs. . . . 28. *B. congesta*

b. Leaf blades tomentose

1. Pubescence of the branches, leaf blades, and flower stalks ferruginous. . . . . 29. *B. lehmanniana*

2. Pubescence of the branches, leaf blades, and flower stalks gray.....30. *B. grisea*
2. Leaf blades dense-pubescent when young, then almost glabrous
  - a. Leaf blades about 2.5 cm. long or less, revolute  
31. *B. myrtifolia*
  - b. Leaf blades about 2.5 cm. and longer, flat  
32. *B. caxamarconsis*
3. Leaf blades glabrous
  - a. Peduncle short, flowers disposed in many-flowered corymb, about 2 cm. in length.....33. *B. coarctata*
  - b. Peduncle long, corymb large, many-flowered, flowers less than 2 cm. long.....34. *B. phillyriaefolia*
  - c. Peduncle very short, flowers disposed in a few-flowered, solitary, subcorymbose raceme, about 4 cm. in length
    1. Bracts shorter than flower stalks.....35. *B. popayana*
    2. Bracts equal to flower stalks.....36. *B. corymbosa*

## I. RACEMOSAE

1. *B. RACEMOSA* Vent. Descr. pl. Cels. pl. 51. 1800; SMALL, North Amer. Fl. 29:36; DC. Prodr. 7:731.—*B. paniculata* Michx. Fl. Bor. Amer. 1:280. pl. 26. 1803.

North America: Florida, dry pine barrens near Jacksonville, June 16, 1894; A. CURTISS 922! and 1728; vicinity of Eustis, Lake County, GEO. V. NASH 803, May 16, 1894; Florida (without locality), RICHARD and COOPER; Cumberland Island, southeastern Georgia; South Carolina; in herb. Gasstromic (Stockholm).

## II. LAEVES

2. *B. SUBSESSILIS* Benth. Plantae Hartwegianae 143; JAMESON, Synops. Pl. Aequat. 2:198; Walp. Rep. 6:420.

Ecuador: Quito, at Mount Loja, JAMESON, HARTWEG 797.

3. *B. DRYMIFOLIA* Linden, ex Hérincq. Revue Hortic. 3:185. 1850.

Colombia (New Granada): In the province of Pampelona, LINDEN 1456. 1847; KARSTEN, Bogota; Venezuela, Silla de Caracas, LINDEN 12, 1842.

The specimens from Silla de Caracas and Bogota are not quite identical with those of Pampelona. They have the inflorescence much shorter and the flower pink.

4. *B. LAEVIS* Benth. Pl. Hartweg. 65; Walp. Rep. 2:730; HEMSLEY, Biolog. Centr. Amer. 2:282. 1881-1882; SMALL, North Amer. Fl. 29:1914.—*B. glabra* Mart. et Galeotti, Bull. Acad. Brux. 1842.

South Mexico: Talaia to Totontepec, HARTWEG 478; Eastern Cordillera of Oaxaca at 5000 ft., GALEOTTI 1811.

5. *B. GHIESBREGHTIANA* (Planchon) nob.—Fruticosa: rami ramulique glabri, vel sparse hispidi: folia lanceolata 6-8 cm. longa, 1.5-1.8 cm. lata, utrinque attenuata, acuta, breviter petiolata, supra minute adpresse tomentosa, vel glabra, subtus glauca semper glabra, integerrima, petiolis glabris: inflorescentia racemosa, subcorymbosa, racemi solitarii, parviflori, apice ramulorum dispositi; pedunculus pedicellique subglabri, juveniles sparse adpresse tomentosi, deinde glabri; pedicelli tenues 3.5 cm. longi, bracteis parvis, deciduis: calyx campanulatus, glaberrimus, lobis 7, ovatis, obtusis, mucronatis margine ciliatis: petala 7 obovata basi attenuata, 2-2.5 cm. longa, 8-9 mm. lata, ad faucem pubescentia: stamina 14, petalis aequilonga, basi dilatata, pilosa: stylus supra calycem incrassatus (in sicco 1.5 mm. latus) stigmatem magno.

Mexico: Province of Oaxaca, M. GHIESBREGHT, 1842-43. Allied to *B. laevis*, but differs in glaucous leaf blades and larger flowers.

6. *B. GLAUCA* Humb. et Bonpl. Aequinox. 2:118. tab.; H.B.K. Nov. Gen. Amer. 3:227; DC. Prodr. 7:731; MORREN, Ann. Soc. Hort. de Gand 1:61; PAXTON and LINDL. Flower Garden 1:84.

Venezuela: Mount Silla de Caracas, H.B.K.; without locality, OTTO 696; TOVAR, FENDLER 743; Colombia or Ecuador, LEHMANN without locality 4964; CALBEREYER 867; Bolivia, bush region of Tres Cruces, north of Santa Cruz, about 1560 m. alt., HERZOG 1611.—A widely distributed species.

### III. GLANDULOSAE

7. *B. CUBENSIS* Griseb. Cat. Pl. Cub. 52; SMALL, North Amer. Fl. 29:35. 1914.

Cuba: Pinar del Rio, western Cuba, WRIGHT 2200.

It is difficult to determine the exact position of this species in the phylogenetic scheme of the species of *Bejaria*. This is due to its scarcity and its local distribution. We have placed it among the Glandulosae because of the glandular hairs covering it. Here it must be placed near *B. mexicana* and *B. discolor*, although very much modified and with highly specialized characters.

But the long, always solitary raceme is very similar to that of *B. racemosa*. We assume, however, that the raceme of *B. cubensis* is a secondary formation, evolved in response to a long isolation and to original conditions. This assumption is based on a high differentiation of the raceme of *B. cubensis*, in comparison with that of *B. racemosa*.

8. *B. DISCOLOR* Benth. Pl. Hartweg. 65; Walp. Rep. 2:730; Hemsl. Biolog. Centr. Amer. 2:282; SMALL, North Amer. Fl. 29:36. 1914; *B. paniculata* Gal. in Herb.; *B. floribunda* Mart. et Gal. in Bull. Acad. Brux. 9:18; *Jurgensenia mexicana* Turcz. in Bull. Soc. Nat. Mosc. 20:151.

South Mexico: Talca, HARTWEG 479; mountains of the eastern Cordillera of Oaxaca, near Villa Alta and Tanatzc, GALEOTTI 1812, 182; Sierra San Pedro Nolasco, JURGENSEN 391, 562; Sille, W. W. SAUNDERS (in herb. Kew Gard.).

This species represents an intermediate stage between *Laeves* and *Glandulosae*, most nearly approaching the species of *Laeves* by the ample foliage, the protraction of the pedicels, and the deciduous pubescence. But the glandular hairs are fairly well developed and deciduous only at the time of fructification, and the calyx and corolla are more pubescent and sometimes viscid as is usual for *Glandulosae*. The specimen of GALEOTTI (1812) described under the name of *B. floribunda* Mart. has a very long and broad inflorescence, and the young leaves are quite pubescent.

9. *B. MEXICANA* Benth. Pl. Hartweg. 15. 1839; HEMSL., Biolog. Centr. Amer. 2:282. 1881-82; Seem. Bot. Voy. "Herald" 317; SMALL, North Amer. Fl. 29:36. 1914.

North Mexico: Sierra Madre, on the road from Mazatlan to Durango, SEEMAN 2130; South Mexico: Balanos, Zacatecas, HARTWEG 69.

10. *B. SPRUCEI* Meissn. in Mart. Fl. Brasil. 7:171.

Eastern Peru, near Tarapoto, SPRUCE 3994 (1855-6).

11. *B. DENTICULATA* Remy, Ann. Sc. Nat. III. 8:236. 1847; WEDDEL Chloris Andina 2:182. 1857; WALPERS, Annals 1:482.

Bolivia: In the province of Yungas at the upper limit of vegetation; on Mount Viscachal, d'Orbigny; Colombia (New Granada), in the province of Socorra, LINDEN 766; FUNCK and SCHLIM 1332.

12. *B. GUIANESIS* Klotzsch in R. Schomb. Reisen in Brit. Guiana 3:1088; N. E. BROWN in Trans. Linn. Soc. 6:46; OLIVER in Trans. Linn. Soc. 2:276. 1887; APPUN, Unter den Tropen 2:232, 287, 292.

We know this species only by the short description given in N. E. BROWN's publication (report on two botanical collections from Mount Roraima in

British Guiana), where it is said to be allied to *B. imthurnii*. It is only with doubt that we determine its position in the scheme of *Bejaria*, although its membership among the *Glandulosae* is evident; Brit. Guiana: Upper slopes and ledge of Mount Roraima, Shomburgh 1041; McCONNELL and QUELSH 40.

13. *B. antioquiae*, sp. n.—Frutex, ramis ramulisque dense tomentosis pilis glanduliferis vestitis: folia breviter sed distincte petiolata, coriacea, rigida, elliptico-oblonga, 4–6 cm. longa, 1.5–2 cm. lata, minute ciliato-denticulata supra glabra et lucida, subtus albo fulvoque pubescentia, glauca, nervo medio glanduloso-ciliato, supra sulcato, subtus prominulo: petioli hispidi: racemi fasciculati 1–4, terminales, densi et multiflori, 7–9 cm. longi, pedicellique ferrugineo-tomentosi et glanduloso-hirti: pedicelli elongati 2–5 cm. longi, bracteis oblongis, pedicellis duplo brevioribus, margine glanduloso ciliatis: calyx campanulatus, 7-lobatus, puberulus, lobis ovatis, obtusis, usque ad medium connatis, biseriatis, margine ciliatis: petala 7 oblonga, obtusa, minute pubescentia, 2 cm. longa: stamina 14, basi dilatata, pilosa, corollae subaequilonga: stylus longe exsertus.

Colombia: Antioquia, inter Yolombo et Cauca, 1500–2000 m., September, 1884, LEHMANN 212.

Allied to *B. imthurnii* N. Brown, from which it differs in having its leaves pubescent beneath, toothed and more elongated, and in having its petals finely pubescent.

14. *B. hispida* Poepp. et Endl. Nova Gen. 1:24; DC. Prodr. 7:731.

Eastern Peru: In the province Huanaco, near Acomajo.

15. *B. LINDENIANA* Hérincq. Rev. Hort. 3:183. 1850; *B. coarctata* Bot. Mag. 5:pl. 4433 (not *B. coarctata* H.B.K.).

Venezuela: from Silla de Caracas to Merida, 1843; LINDEN 387, Merida; Moritz 365, 1844–45.

The species published in Bot. Mag. (pl. 4433) is totally different from what is described by HUMBOLDT and BONPLAND under the same name. This error was pointed out by HÉRINCQ, who called it *B. lindeniana*, in compliment to LINDEN, the horticulturist and collector. The discussion of this species showed the great difference between *B. coarctata* H.B.K. and that of Bot. Mag., a difference necessitating their placement in different sections.

16. *B. AESTUANS* Mut. ex Linn. Mant. 242, suppl. 247; Sp. Pl. 2:847; H.B.K. Nov. Gen. Am. 3:229; Humb. et Bonpl. Pl. aequi-

nox., 120; DC. Prodr. 7:731; Vent., Choix n. 52 in adn.; JAMESON Synops. pl. aequat. 197; VAN HOUTTE, Fl. des Serres 4:3288.—*B. oblonga* Pers. Ench. 2; *Acunna oblonga* Ruiz et Pav. Fl. per. syst. 123.

Colombia (New Granada): Santa Fé de Bogota, near Gonzanama, Mutis; from the type locality, FUNCK and SCHLIM 1604; Venezuela, FUNCK and SCHLIM 874; Merida; Ecuador; Quito, Mount Loja, JAMESON.

17. *B. DENSA* Planch. ex Hérincq, Rev. Hort. 4:185; *B. microphylla* Planch. ex Paxt. et Lindl. Flower Gard. 1:86; *B. albiflora* Linden in Hb. Vindobon.

Colombia (New Granada): Province of Pamplona, SCHLIM 1721; Chapo, 2400 m., LINDEN 13511.

18. ~~B.~~ IMTHURNII N. BROWN. Trans. Linn. Soc. 7:46; *Befaria* aff. *resinosae* Mutis, OLIVER in Trans. Linn. Soc. Bot. 2:278. 1887.

British Guiana: Summit of Mount Roraima, 8600 ft., THURN 310; McCONNELL and QUELSH 94, 146.

19. *B. resinosa* Mutis, Ic. Am. ex Linn. Suppl. 246; DC. Prodr. 2:731; Vent. Choix t. 52; PERSON, Synops. 3:1807; *Acunna lanceolata* Ruiz et Pav. Syst. Fl. Per. 123; *B. resinosa*, *B. lanceolata* G. Don Gen. Syst. 3:849.

Colombia: Santa Fé de Bogota, MUTIS. Santa Marta, H. SMITH 1406; Peru: on the high hills of Rodos Pillao and Huassa.

20. *B. LEDIFOLIA* Humb. et Bonpl. Pl. Aequinox. 2:124; H.B.K. Nova Gen. Am. 3:229; DC. Prodr. 2:731; VAN HOUTTE Fl. de Grochshor. 3:t. 3.

Venezuela: Silla de Caracas, LINDEN 1281; FUNCK 313; Colombia: MORITZ 365.

#### IV. EGLANDULOSAE

##### RACEMOSAE

21. *B. PALLENS* Remy in Ann. Sci. Nat. 8: 229. 1847; Walpers Annals 1:842.

Bolivia: In province Yungas on the way from Chilca to Chulmani (d'Orbigny), ESPIRITO SANTO BANG 4167.

22. *B. MATHEWSII* Field, Bot. Mag. t. 4081.

Peru: Province Jaulia, MATHEWS 14241; province Chachapoyas, MATHEWS 1426.

23. **B. subserrata**, sp. n.—Frutex: rami minute tomentosi, ramulis hispidis: folia alterna, coriacea, rigida, oblonga, 3 cm. longa et 1-2 lata, apice obtusa vel acuta, basi in petiolum brevem angustata, supra lucida et glabra, subtus glauca albo-fulvoque pubescentia, margine denticulato-ciliata, nervo medio supra sulcato, subtus prominulo, adpresse tomentoso: inflorescentia racemosa, subcorymbosa, racemis 2 apice ramulorum dispositis: pedunculus pedicellique ferrugineo-tomentosi, pilis longis et glandulosis patenter vestiti: pedicelli tenues 2.5-3 cm. longi, bracteis caducis: calyx campanulatus, dense pubescens, 7-lobatus, lobis late ovatis, obtusis: petala 7, oblongo cuneata, apice rotundata, pubescentia: stamina 14 libera, basi pilosa, dilatata et petalis 2 cm. longis vix longiora: stylus exsertus, stigmatibus magno.

Venezuela: Without precise locality, FUNCK and SCHLIM 1331.

Allied to *B. denticulata*, which differs in its paniced inflorescence and in absence of glandular hairs on the pedicels and peduncles; from *B. caxamarcensis* it differs in its minutely toothed leaves and in the dimension of the flowers.

24. **B. boliviensis**, sp. n.—Frutex: rami glabri vel pulverulenti, ramulis hispidis: folia alterna, coriacea, rigida, oblonga, 3-4 cm. longa et 1.2-1.8 cm. lata, basi angustata, apice obtusa vel acuta, saepe obtuse mucronata, petiolata, supra lucida et glabra, nervo medio basi tomentosa, subtus glauca albo fulveque tomentosa, nervo medio setoso; petiolis hispidis: inflorescentia dense paniculata ramosa, pedunculis pedicellisque patenter ferrugineo-pilosis, pilis longis, eglandulosis: pedicelli ca. 2-2.5 cm. longi, crassi; bracteis foliis non dissimilibus hispidissimis, deciduis, margine denticulatis, quam pedicellis triplo brevioribus: calyx campanulatus, 7-lobatus, pubescens, lobis ovatis, obtusis: petala 7 oblonga, obtusa, juvenilia pubescentia deinde glabra, 1-1.3 cm. longa: stamina 14 (-15) usque ad medium pubescentia, basi dilatata, brevissime inter se connata: stylus crassus exsertus.

Bolivia: "From 1500 to 2000 miles in the interior, lat. 15-18° south," BRIDGES 177; MANDON 563!.

Allied to *B. subserrata*, but differs in its inflorescence and entire leaves.

25. **B. GRANDIFLORA** H.B.K. Nova Gen. 3: 228; Humb. et Bonpl. Pl. Aequinox. 2: 122; DC. Prodr. 2: 731; JAMESON Synops. pl. Aequator. 2: 197. 1865.

Ecuador: Quito; in the province of Loja, Paramo de Saraguru, Humb. and Bonpl.; JAMESON; HARTWEG 716: Venezuela, without locality, FUNCK and SCHLIM (1847) 1329! Colombia: Cauca, Popayan (1884) Lehmann 220.

26. *B. PARVIFLORA* Benth. Pl. Hartweg. 143; Walp. Rep. 6:420; JAMESON Synops. Pl. Aequat. 2:197.

Ecuador: Quito, at Mount Loja, JAMESON; HARTWEG 795; Colombia: Without locality, MORITZ 478; St. Martha (1844) PURDIE (in herb. Stockholm).

27. *B. DECORA* Drake in Morot, Jour. de Bot. 3:71. 1889.

Ecuador: Chonta-Cruz, POORTMANN 116: Colombia: In the province of Rio Hacha, LINDEN 1618.

#### CORYMBOSAE

28. *B. congesta*, sp. n., Frutex: rami cinerei, raro pilosi, ramulis hispidis canis, dense foliosis: folia conferta, coriacea, rigida, valde revoluta, margine serrato-ciliata, utrinque hispida, setis longis basi tuberculatis vestita, lanceolata, 5 mm. lata, 15 mm. longa, basi rotundata, apice acuminata, acuta, nervo medio supra impresso (subtus prominulo, setoso) avenia, breviter petiolata, petiolis hispidis: racemi subcorymbosi densi, multiflori, pedunculis pedicellisque ferrugineo-hirtis: pedunculi breves, pilosi, crassi, pedicellis breves, 5–8 mm. longis, calyce paulo longioribus: bracteae binae ad pedicelli basin dispositae, lineares, pilosae: calyx campanulatus petalis quintuplo brevior, 7-lobatus, pubescens, lobis ovatis, obtusis, fere usque ad basin liberis, biseriatis, 5 mm. longis, 3 mm. latis: flores magni, petalis 7 oblongis, obtusis, 2.5 cm. longis, 5–7 mm. latis, apice pubescentibus: stamina 14 basi dilatata et pilosa, petalis subaequilonga: Stylus exsertus crassus, stigmathe magno capitato: ovarium septisulcatum.

Venezuela: Without precise locality, FUNCK and SCHLIM. Allied to *B. myrtifolia*, but differs in its obtuse calyx lobes, stout and short pedicels and small bracts.

29. *B. lehmanniana*, sp. n.—Fruticosa: rami cinereo-pilosi, ramulis ferrugineo-tomentosis, dense foliosis: folia conferta, coriacea, lanceolata 1.5–2 cm. longa, utrinque attenuata, petiolata, margine revoluta, supra lucida et cinereo- et ferrugineo-hispida, subtus dense ferrugineo-tomentosa, petiolis hispidis: racemi brevissimi, subcorymbosi, densi, pedunculis pedicellisque ferrugineo-tomentosis, pedicellis crassis circa 1–1.2 cm. longis, bracteis forma foliis valde



similibus: calyx campanulatus, amplus, 7-lobatus, 6 mm. longus, pubescens, lobis angustis, acutis, quam petalis 8-plo brevioribus, biseriatis: petala 7 oblonga, obovata, obtusa, apice (vel juvenilia omnino) pubescentia, 0.8–0.9 cm. lata, 3.5–4 cm. longa: stamina 14 basi dilatata pilosa, petalis vix longiora: stylus crassus, longe exsertus; stigmatibus 7-partito magno.

Colombia vel Ecuador, loco non indicato, LEHMANN 4962.

A very distinct species, perhaps nearer to *B. cinnamomea* than to any other; from this uncertain species it differs in the form of inflorescence and in its much larger flowers.

30. *B. grisea*, sp. n.—Frutex: rami ramulique griseo-tomentosi: folia approximata coriacea, rigida, elliptica, basi rotundata in petiolum vix attenuata, apice obtusiuscula, semper brevissime et obtuse mucronata, 20 mm. longa et 12 mm. lata, supra lucida, hispida vel glabriuscula, subtus glauca, dense albofulvoque pubescentia, margine leviter revoluta et integerrima: racemi terminales, simplices, subcorymbosi, laxi, parviflori, pedicellis crassis adpresse griseo- vel ferrugineo-tomentosis, 1.5 cm. longis; bracteis foliiformibus pedicelli  $\frac{3}{4}$  aequalibus: calyx campanulatus amplus, 7-lobatus, lobis latis, ovatis, obtusis, pilosis, omnino liberis, 6 mm. longis et 4 mm. latis, quam petalis 5-plo brevioribus: petala 7 oblonga, obovata, apice obtusa et pubescentia, 2.5 cm. longa, 8 mm. lata: stamina 14 basi dilatata pilosa: stylus crassus, exsertus, stigmatibus magno.

Colombia (New Granada): Without precise locality, FUNCK and SCHLIM 14751. A very distinct species, unlike any other.

31. *B. CAXAMARCENSIS* H.B.K. Nova Gen. 3:230; DC. Prodr. 6:731.

Peru: In the Andes of Caxamarca, HUMBOLDT.

32. *B. MYRTIFOLIA* Hérincq in Rev. Hort. 4:182. 1857.

Colombia (New Granada): Province of Bogota, LINDEN 773; KARSTEN.

33. *B. COARCTATA* H.B. Pl. Aequin. 2:125; H.B.K. Nova Gen. Am. 3:228; DC. Prodr. 7:731; Wedd. Chloris And. 2:183. 1857; Walpers Ann. 2:1123; Paxt. et Lindl., Flower Gard. 1:83; VAN HOUTTE, Fl. des Serres 4:3286; non *B. coarctata*. Hook. Bot. Mag. t. 4433; *Acunna lanceolata* Cav. ex DC. Prodr. 7:731.

Peru: Paramo de Janaguanga, near the town Caxamarca; Colombia: Pasto, at the Borboncillo, February, 1881, LEHMANN 564; without precise locality, FUNCK and SCHLIM 1600.

34. *B. phillyriaefolia* Benth. Pl. Hartweg. 225; Walpers Rept. 6:420; Wedd. Chloris And. 2:182. 1857.

Colombia: Province of Popayan, HARTWEG 1231; Cauca, 1700 m. alt., Aug. 16, 1881, LEHMANN 823.

35. *B. popayana*, sp. n.—Frutex, ramis ramulisque tomentulosis: folia conferta, coriacea, rigida, oblongo-elliptica, obtusiuscula vel acuta, apice breviter calloso-mucronata, distincte petiolata, 3–3.5 cm. longa, venis distinctis perspicuis, nervo medio supra sulcato et villosa, subtus prominulo et parce tomentoso: inflorescentiae racemus subcorymbosus, solitarius, apice ramulorum dispositus, brevis, parviflorus, floribus magnis 5–8: pedunculus subnullus pedicellique ferrugineo-tomentosi, pedicelli parvi 8–10 mm. longi, bracteis linearibus, 3–5 mm. longis, ferrugineis: calyx campanulatus, amplus, 7-lobatus, tomentosus vel subglaber, lobis late ovatis, obtusis, margine ciliatis: petala 7 omnino pubescentia, 4 cm. longa, circa 1 cm. lata, oblonga, apice rotundata: stamina 14 libera, basi dilatata pilosa, petala  $\frac{1}{4}$  excedentes: stylus longissime exserta, stigmatibus magno, 7-partito.

Colombia: Popayan, KARSTEN.

Allied to *B. phillyriaefolia*, from which it differs in the pubescence of the petals, in dimension of the flowers, and in the very short peduncle.

36. *B. corymbosa*, sp. n.—Frutex, ramis ramulisque tomentulosis: Folia coriacea, rigida, oblonga, apice obtusiuscula, brevissime callosa, distincte petiolata, 3 cm. longa, 1–1.5 cm. lata, glabra, subtus glauca, supra lucida, venis distinctis, perspicuis, nervo medio supra sulcato et villosa, subtus prominulo et parce tomentoso. Inflorescentiae corymbosae corymbus solitarius, apice ramulorum dispositus, brevis, parviflorus, floribus magnis: pedunculus brevis pedicellique ferrugineo-tomentosi, pedicelli 1–1.5 cm. longi; bracteis lanceolatis foliiformibus pedicello aequantibus: calyx campanulatus, amplus, 7-lobatus, breviter ferrugineo-pilosus, lobis late, ovatis, apice attenuatis, margine ciliatis: petala 7 glabra, margine ciliata, 2 cm. longa, 0.7 cm. lata, oblonga, apice rotundata: stamina 14 libera, basi dilatata pilosa, petala  $\frac{1}{4}$  excedentes: stylus exsertus, stigmatibus magno 7-partito.

Colombia: Pasto, Venta Menes, KARSTEN.

Allied to *B. popayana*, from which it differs as to its glabrous petals and in the dimension of its bracts, which are equal to the pedicels.

## IMPERFECTLY KNOWN SPECIES

37. *B. CINNAMOMEA* Lindl. in Garden. Chron. 1848.—We have no specimen of this species in our herbarium, and we know it only by the short description given in Flow. Gard. 1:86.<sup>4</sup> It seems to be near to *B. lehmanniana*, which differs by its large flowers and corymbose inflorescence.

38. *B. SCHOMBURGKIANA* Klotzsch in Schomb. Reisen in British Guiana 3:1088; BROWN in Trans. Linn. Soc. 6:10. In both works only the name is mentioned.

Guiana, Humiruda, and southern declivity of Mount Roraima 6000 ft., R. SCHOMBURGK.

39. *B. TRICOLOR* Linden ex Hérincq, Rev. Hortic. 4:185.—This is the diagnosis given by HÉRINCQ: "The plant has the branches ferrugineous and the leaves blazing. The flowers are very pretty, white and pink with yellow spots at the base of the petals." It is too short to permit of definite conclusions as to the precise status of *B. tricolor* in the system of Bejarias.

## DOUBTFUL SPECIES

40. *B. FLORIDANA* Gdgr. Sertum plantarum novarum in Bull. Soc. Bot. France 65:56. 1918.—The description given by GANDOGER is too brief and appears to be only a comparison with *B. racemosa*: "a *B. racemosa* recidit ramis novellis non hispidis, foliis oblongis majoribus, pedunculus viscosus bracteis multo brevioribus fructuque magis depresso. Folia coriacea, glauca integerrima." The author states it to be more frequent than *B. racemosa*. It is possible that this species is only a variation of *B. racemosa*, but we feel certain that in many respects it differs decidedly from *B. racemosa*.

41. *B. PARVIFOLIA* Rusby, Bull. N.Y. Bot. Gard. 8:110. 1912.—We cannot detect the difference between this species and *B. glauca* H. et B. We have not seen the specimen collected by WILLIAMS, but the description is quite identical with the one by HUMBOLDT.

JARDIN BOTANIQUE PRINCIPAL  
LENINGRAD, U.S.S.R.

<sup>4</sup> "Branches downy and hispid; leaves slightly downy above covered beneath with thick ferruginous wool. Flowers in a close terminal panicle, with very woolly and hispid stalks, and calyces. Its flowers are smaller than in *B. aestuans*, and are arranged in a close panicle."

# ANATOMY OF LYCOPODIUM SPORELING

EARLE AUGUSTUS SPESSARD

(WITH PLATES IX, X, AND NINE FIGURES)

## Material

The material of *Lycopodium lucidulum* Michx., on which the following account is based, was found in the vicinity of Marquette, Michigan in 1918. Several hundred sporelings of this species, ranging in length from 1 mm. to 4 cm., comprise the collection. So far as can be determined, these sporelings were not more than two years old. This series appears to be complete enough to show all the stages of growth between the embryo and the typical adult colony, but such is not the case. It is not difficult to find young colonies of *L. lucidulum* with the individual plants no longer than the sporelings mentioned, but close examination shows that these plants are derived from the well known vegetative gemmae shed in great numbers by many adult plants. One gemmaling may give rise to an entire colony of adults, through branching and a subsequent fragmentation of the stoloniferous stem at its base. In view of this fact, the series of sporelings found seems complete enough to establish a connection between sporeling and adult, but since this connection was not made, an explanation must be attempted.

The most obvious explanation for the discrepancy would be that the later stages were merely overlooked, and this would be entirely justifiable were there no other facts to be considered. Briefly, then, a number of observations will be mentioned. First, the shape and color of the adult plants and gemmalings are the same. The sporeling is lighter in color, and the diameter of the stem tapers from the apex downward to the foot and roots. The sporeling, even at its greatest length, is a much more delicate plant than the gemmaling, which is stockily built. Second, the sporelings grow in drier ground than do either the gemmalings or the adults, and they die in great numbers because there is not enough moisture. The gemmalings almost invariably die also when they germinate in the drier places where

sporelings are found. On the other hand, the gemmalings thrive in the moister ground, where the sporelings find it difficult if not impossible to mature.

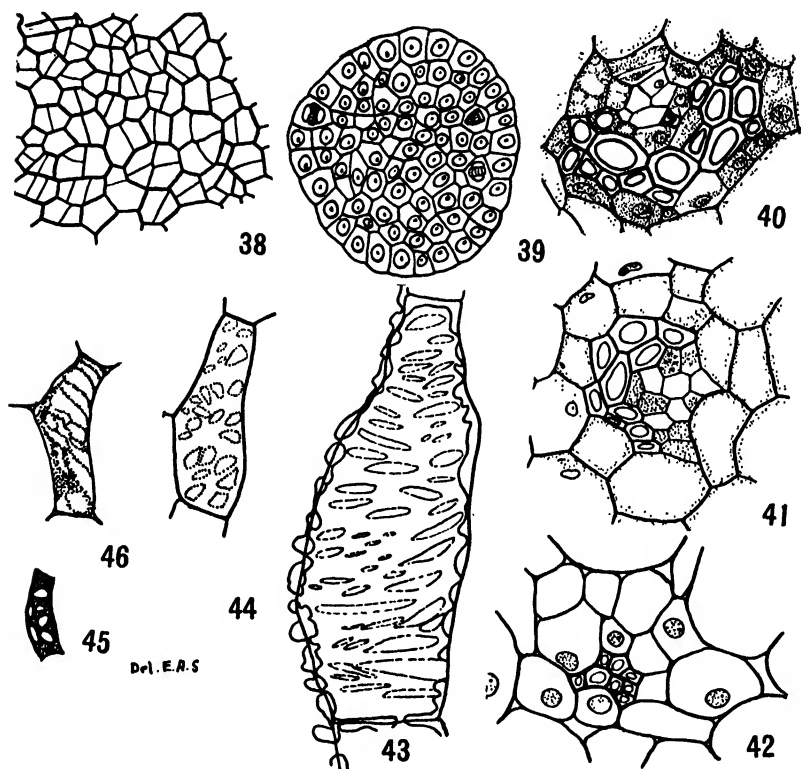
These observations force the conclusion that *L. lucidulum* is almost if not quite ecologically incapable of using the sexual process for survival. Physiologically the plant is entirely efficient sexually; but the products of the process, the sporelings, die because they cannot survive the environment. The adult colonies of *L. lucidulum* are rarely if ever the result of sexual reproduction. In the experience of the writer the transition from sporeling to adult has never occurred. In the following description of the anatomy of the sporeling of this species of *Lycopodium*, it will be clear to those acquainted with the stele of the adult plant that there are many points of difference. The writer is now investigating the anatomy of the gemmalings in the hope that some of the discrepancies may be accounted for.

### Stele

The apical meristem extends downward in the stem to a distance of about  $70\mu$ . It is composed of an actively dividing epidermis and an inner fundamental tissue. The whole may be further divided into an apical group extending down to the  $15\mu$  level, and a subapical group extending downward to the  $70\mu$  level. In the apical group (fig. 38) the cells divide in three planes in both the epidermal and fundamental regions. In the subapical group (fig. 39) the divisions are in three planes in the epidermis, but largely in two planes in the fundamental region. There is dermatogen, but no plerome-periblem group. Histologically the stele arises as a direct modification of the cells of the fundamental region. The cortex is derived from the same tissue simply through not becoming differentiated. In none of the sporelings was the writer able to demonstrate an endodermis, although a very definite demarcation exists between cortex and stele. At a level of about  $80\mu$  the entire fundamental region, with the exception of a few cells at the periphery, stains as a desmogen strand. The cortex of the stem lower down appears to have been derived largely from the dermatogen and less from the fundamental region.

The adult stele consists of pericycle, xylem, phloem, and pith. The cells of the pericycle are elongated and contain protoplasm.

The left hand cell with nucleus in fig. 25 is from the pericycle of a stem. The end walls of cells lying within the pericycle usually are



FIGS. 38-46.—Fig. 38, section of stem 15  $\mu$  from tip; fig. 39, section of same stem as in fig. 38, 70  $\mu$  from tip; fig. 40, section of same stem just above primary root and foot; fig. 41, section of root of same plant just below foot; fig. 42, section of leaf; fig. 43, foot cell of sporophyte; fig. 44, prothallial cell from conducting region and near foot; fig. 45, portion of phloem cell in sporophyte stele near foot; fig. 46, young xylem cell; figs. 38, 39,  $\times 350$ ; figs. 40-46,  $\times 775$ .

diagonal, while those of the pericycle usually are not. Figs. 28 and 30 show the extent of the pericycle. In the former the xylem abuts on the cortex in some places. The pericycle cannot be considered as a definite sheath inclosing the balance of the stele.

The major portion of the stele is occupied by xylem. Figs. 1-24 show this element in heavy black, with the limits of the stele proper

indicated by a broken line. The extent of the xylem is shown for the stem in figs. 25, 27, and 28; for the root in figs. 31-33; for the leaf in fig. 42.

The preparations from which figs. 28, 30, and 32 were taken to illustrate phloem are so unsatisfactory that this element of the stele must be left for further investigation. That these thin-walled cells are phloem I am certain, but the details of their structure are doubtful. The phloem groups lie on radii and on a circumference different from those on which the protoxylem strands arise. In *L. lucidulum* phloem is not only less extensive than in higher plants, but its differentiation seems to be delayed.

The word pith has been used here for want of a better term. A glance at figs. 26 and 28 shows a group of large elongated cells within the stele, which were derived from the fundamental region of the subapical group of cells, and which have not differentiated into any stelar element to which they can be referred. If this plant could have been examined after it had grown several more years, perhaps we should find these cells differentiated into xylem or phloem. The cells are stelar in origin, are to be considered as stelar elements, and in view of what will be said about them in the discussion at the end of this paper, will be called pith.

### Leaf and stem

TURNER has shown for the adult of *Lycopodium*, and STOVER for grasses, that the leaf strand is first differentiated in the leaf itself, and later makes connection with the stem group. My preparations confirm this in the juvenile stages of *L. lucidulum*. While the phyllotaxy of this species is not constant, there exists a very definite relationship between the leaf trace and the locus of metaxylemic differentiation. This interesting feature will be presented in a separate paper.

The protoxylem of the stem is laid down on four, five, or six radii, as shown in fig. 28. The metaxylem develops centripetally, and in a very regular sequence of units related to the leaf traces. The mass of metaxylem appears to be very irregular, as shown in figs. 1-24. This irregularity is due to the presence of adventitious roots.

### Primary root

The stele of this organ is essentially like that of the stem, except that there is not the disturbance due to leaf and adventitious root formation. Fig. 24, from the primary root of an old sporeling, may be compared with fig. 32 from the middle stem region of a young sporeling. The metaxylem of the two strands arises by a process of differentiation between two protoxylem strands. Why the root xylem should appear similar to the stem xylem in form is difficult to explain. The stem metaxylem is laid down in definite relation to the leaf traces, but the same pattern results in the primary root, where there are no leaves.

### Adventitious roots

These roots are very remarkable. They were called internal roots by STRASBURGER, and were studied by Miss STOKEY in *L. pithyoides*. A complete bibliography of the work done previously will be found in her paper. While not as abundant as in *L. pithyoides*, they are plentiful enough to give the primary root of *L. lucidulum* only temporary significance. They arise sometimes within a millimeter of the stem tip, absorb the cortex of the stem, and push their way down through it to within a millimeter of the primary root and the foot of the sporeling. There they emerge and pass into the soil. An adult plant and a large sporeling have masses of these roots at the base. If, every time a new adventitious root appeared, we were to amputate all of the plant but it and the region above it, we should see a plant not unlike the very young sporeling with a primary root. The whole process simulates a condition which looks like a perpetual recapitulation of the early sporeling stage.

The adventitious root begins as a proliferation of pericycle cells. A cap is produced normally. When about half a millimeter long the stele is clearly differentiable. The subsequent xylem of this new root finally connects with the neighboring edges of two xylem crescents in the stem (fig. 14), or, more frequently, with the ends of a single crescent (fig. 35). The stem xylem does not contribute to the xylem of the root. It is very easy to fancy that we observe the stem xylem bending downward and outward to enter the root, but close



examination shows that between the mature stem and root xylem there lies an immature group of cells in the process of being transformed into xylem. Figs. 1-22 show the origins of five adventitious roots in a typical sporeling. The crescents of xylem in the stem are constantly being shifted about. If the stem xylem were removed bodily, it would show a very ragged appearance. In any given vertical line there are many gaps in the xylem, places where the fundamental stelar tissue has not formed xylem. These are not leaf gaps, which are definite in their positions; they are places where no differentiation into specialized xylem has occurred.

Leaf, stem, and root develop their xylem *in loco*, and later connect. The sequence of events is the same in each case. From an acropetal meristem there is developed a primary stele, which, in the case of the stem, occupies nearly the entire diameter. From this as a beginning, the types of stele which may develop are either a radial, a collateral, or a concentric.

### Conducting parenchyma

The cortical cells of the root and stem have thick walls with pits. In some cells whose walls stain deeply with anilin blue, smaller white slits appear under a magnification of about 1500 diameters. It is doubtful whether these are perforations entirely through the wall, although the technique used has made it appear so. The inner cells of the foot (fig. 43), the epidermal cells, and even the cells of the prothallium into which the foot projects, all have pitted walls. The epidermal cells of the foot are also glandular and contain starch.

From the stele of the stem at the foot region, through the foot itself, thence into and through the central region of the prothallium, there extends a system of conducting cells provided with pits. Below this lies the fungus region. This relationship is not without significance. From soil to sporeling there is a direct line of communication in the form of a conducting mechanism adequate for the translocation of food.

Several things are known regarding the situation: (1) The prothallium seems to require the fungus, for it will not grow without it in the cases examined. (2) Carbohydrate is stored in prothallia entirely devoid of chlorophyll. Whether this carbohydrate comes from

the soil as such, or is synthesized from materials brought into the prothallium by the fungus, is not known; that the source is in this direction is hardly doubtful. It is important to mention that in epiphytic and surface forms that possess some chlorophyll in the prothallia, there is a less extensive invasion of the fungus. (3) The sporeling uses this supply of food, together with the entire protein content of the prothallium, absorption taking place through the foot. This absorption is so complete that prothallia are often found consisting only of the epidermal hull, the fungus lining it, and the sporeling foot still adhering to the inner portion of the withered mass. An excellent confirmation of this was found in the sporeling shown in fig. 37. This sporeling possessed no primary root whatsoever, and therefore had to reach its state of maturity entirely at the expense of the prothallium. Adventitious roots had developed within the cortex, but none had broken through it to reach the soil.

Here, then, is a definite symbiotic requirement with a definite anatomical mechanism to meet it: the fungus supplies the prothallium, and the prothallium supplies the sporeling with food. Until the chemical changes between the three organisms are determined, it cannot be stated whether this is a case of parasitism or of symbiosis; I have merely attempted to show that there is a mechanism present for the transport of materials from soil to sporeling.

### Discussion

The writer has purposely avoided speaking literally of the "fusion" and the "splitting" of xylem, since it is doubtful whether these terms can be used with any degree of accuracy here. When xylem develops at one place and fails to develop at another, as shown in figs. 6 and 7, a condition is presented which makes it appear that splitting has occurred; or, as in figs. 8 and 9, that fusion has taken place. If a clay model were made of the stele of *L. lucidulum*, with xylem shown in red, and the fundamental stelar tissue shown in blue as a background, many blue patches would appear where the xylem failed to appear. These patches are areas of arrestation. Xylem cells do not shift about; they are differentiated at the spots where the cells from which they are transformed were laid down. In places where these cells are not transformed we have

merely to do with an omission, and not with a splitting. This is a polyxylic phenomenon, and might conceivably have some bearing on the matter of polystely, but it is not a phenomenon brought about by the breaking up of an antecedent stele; it is due to the omission of xylem activation, whatever may be the cause of that.

The history of vascular ontogeny may be interpreted from both ends of a plant at the same time. Undifferentiated meristem changes into elongated parenchyma, and this into prosenchyma. This is a longitudinal differentiation. Beyond this point differentiation is strictly lateral, except where longitudinal differentiation is renewed in the formation of adventitious roots or of lateral buds. Lateral differentiation takes place on definite circumferences and on certain radii. Definite regions of the pre-stele are to become xylem, phloem, pericycle, or none of these. I wish to make it clear that xylem, for instance, is not to be interpreted as an errant invader capable of splitting and of growing in several directions; it is a fixed stage in ontogeny. The early protoxylem becomes a significant point of reference, not merely to the anatomist but to anatomy, for the loci of the early elements must be radial, and may be near or away from the center of the stele, furnishing the starting points for exarch, mesarch, or endarch structures. This variation in the radial location of protoxylem is the critical phase in stelar ontogeny. After it is established, it is a matter of indifference as to the type of stele capable of being developed from it. In the sporeling of *L. lucidulum* several phases are encountered. A radial protostele passes into a collateral protostele; this in turn may become a concentric protostele with internal phloem, an amphivasal protostele. Again, it may pass into a monocollateral or a bicollateral protostele. Here is a vascular "No man's land." This is precisely what we should expect in *Lycopodium*, since the genus is an ancient one. It stands at the cross roads of stelar development, unable to advance.

The sporeling of *L. lucidulum* offers some evidence concerning the origin of pith. JEFFREY claims that this tissue is cortical in origin; VAN TIEGHEM, BOWER, and others claim that it is stelar; this investigation confirms the latter view.

It was mentioned in a previous paragraph that where xylem does not differentiate in the stelar tissue there remains a gap. The xylem shifts with regard to our view of it, but it neither moves its position

nor splits. These gaps are present before and after the appearance of adventitious roots. The cells in these gaps are prosenchymatous, that is, stelar in nature. The gaps are not holes left by a moving xylem to be filled in by an accommodating cortex.

So long as a cell has not lost its plasticity, we may expect to observe it returning to the functional behavior of its meristem forbear. There comes a time in the process of differentiation, however, when it is impossible for the cell to turn back; it is fixed as it were. The cell may be stopped in its development, and thus fail to complete a destiny; it may turn at some stage from one function to another, but the new function must lie within the limits of the plasticity of the protoplasm of the cell at that moment. Herein lies the difficulty in attempting to interpret the pith as cortical in origin. Cells from the outer circumferences of the cortex and of the stele do become meristematic to the degree of producing new stems, leaves, and roots. In all cases, however, these cells are not extremely removed from meristem of some nature lineally. At the younger levels, where such a phenomenon as a cortical invasion would have to take place, the stelar cells are as plastic as the cortical. At the lower levels, where the xylem has differentiated to leave the gaps, the cortex cells have thick cell walls, and have less protoplasm than is seen in meristematic tissue. It is doubtful whether we can point to definite cells of the subapical region in *L. lucidulum* and assign to them a certain destiny. It may be true that physiologically it is a matter of indifference to the cells, but as differentiation proceeds, this indifference decreases or becomes less pronounced. The ontogenetically older cells are less plastic; those in the cortex are cortex and those in the stele are stele. The ability to return to the meristem condition has disappeared. If we were to imagine that pith is derived from cortex, we are faced with the phenomenon of a fully differentiated structure invading another fully differentiated one, and becoming an integral part of that structure without creating the least physical disturbance. Roots have been seen to invade cortex, however, and this invasion is announced by a great display of cortical disintegration. There is no doubt to the observer that an invasion is taking place, but when we try to imagine a cortex invading a gap left in the stele, we find it too orderly to evade suspicion.

The word gap is a good one, implying a place where something

has been left out, while a hole implies that something has been taken out. Cortex was never left out or taken out of a stelar gap, for cortex was never there. Undifferentiated stelar elements were there from rather early ontogeny. It is more sensible to interpret the gap as a spot of potential xylem, which, not yet past its period of plasticity, was called from one function to another. It follows naturally that if the gaps are stelar in origin, the pith cannot be cortical in origin. As potential stele, the pith is that portion in the stelar region which is plastically indifferent as to its future. At some place in ontogeny, physiological requirement demands that it be not vascular at all. It does not cease to be conductive, it never reaches that state. This interpretation does away with the mechanical difficulties involved when we try to imagine one tissue invading another. Furthermore, if pith is not potential stele, we are presented with an anatomical paradox in the isolated conductive strands sometimes found in pith.

The writer wishes to express his thanks to Professor W. J. G. LAND of the University of Chicago for his criticisms in the preparation of this paper.

### Summary

1. The stele of the sporeling of *Lycopodium lucidulum* is a protosteles which is never solid, but may have the elements arranged concentrically or collaterally.

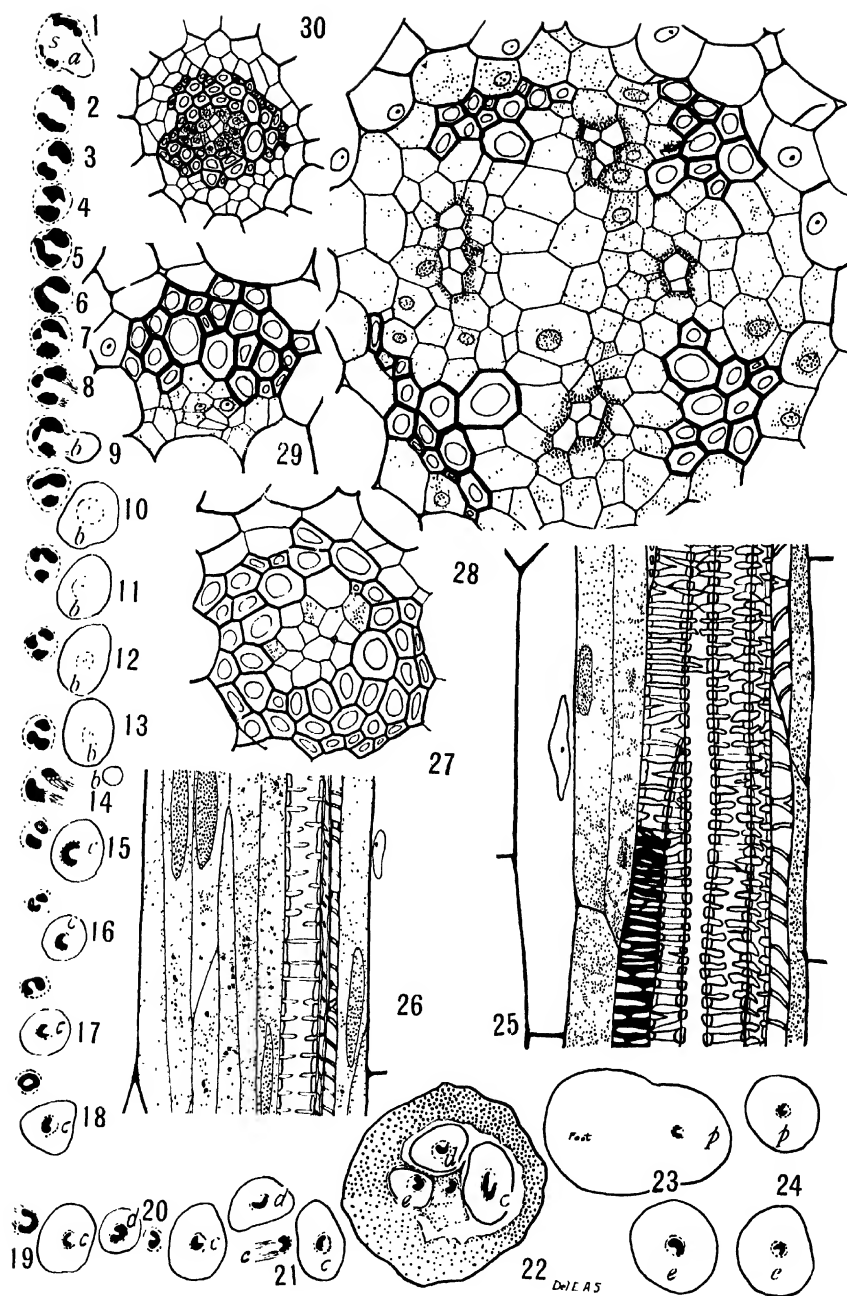
2. There is no well defined endodermis. The phloem is not very far advanced, has not been worked out carefully, but appears to be at first radial and later less definite.

3. The xylem is radially arranged at first, and later forms crescent-shaped slabs or a complete ring.

4. The xylem is developed independently in root, leaf, and stem; these are later joined.

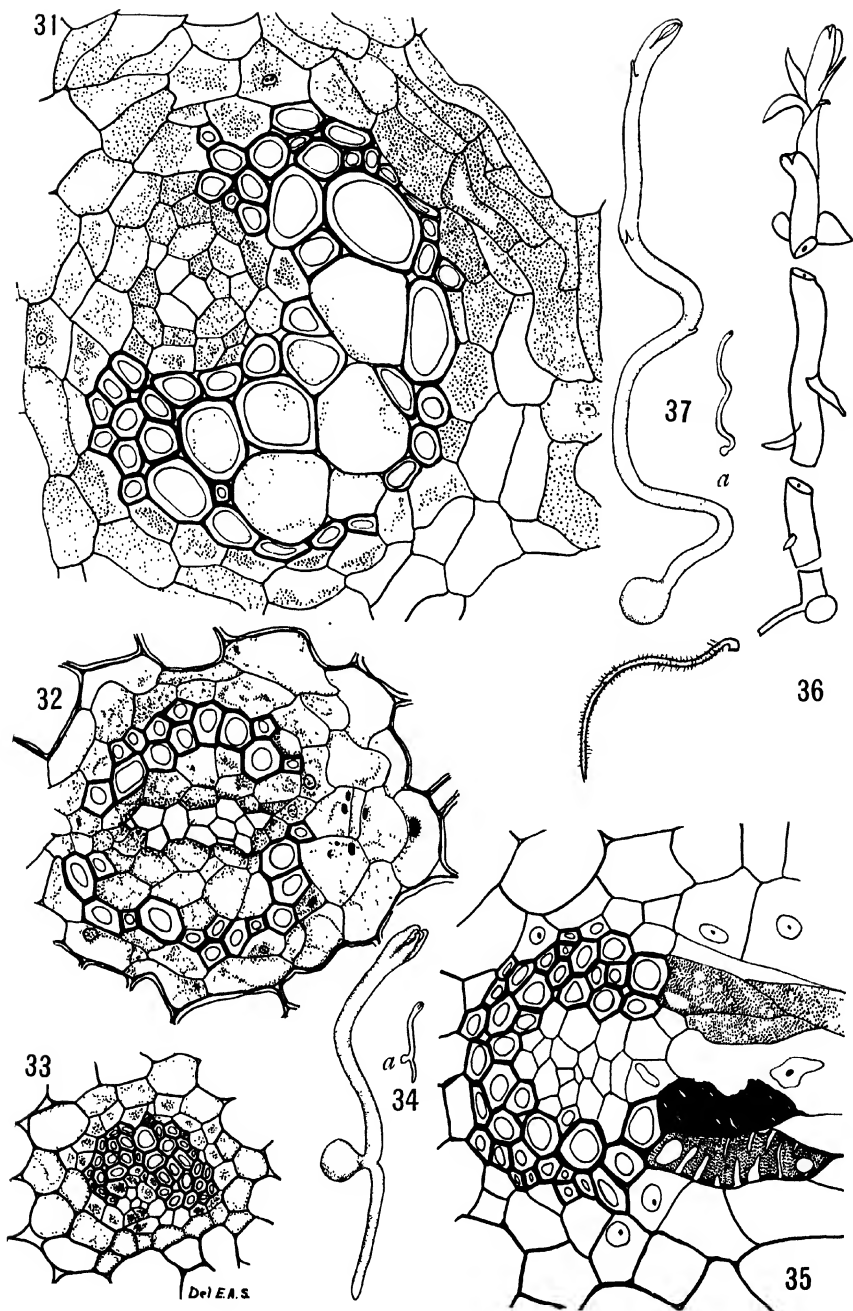
5. There is no splitting or fusion of xylem in a literal sense.

6. Gaps in the xylem are interpreted as not due to an invasion of cortex, but as an arrestation in early ontogeny, causing the cells of the stele to be diverted from one function to another. Pith is put in the same category.



SPESSARD on LYCOPODIUM









7. Cortex and stele are differentiated almost if not quite as far back as the initials.

8. The phyllotaxy varies with individuals, and has an apparent determinative influence on the form of the stem xylem.

9. The adventitious roots appear acropetally, and pass down through the cortex, emerging near the foot.

10. There is an adequate conducting apparatus from the stele of the sporeling to the fungus region of the prothallium. The sporeling may derive some of its food through the endophytic fungus by way of the prothallium.

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## DESCRIPTION OF PLATES IX, X

### PLATE IX

FIGS. 1-24.—Sections of stele of stem and adventitious roots, from point a few millimeters below stem tip down to about middle of primary root: *s*, stele of stem with xylem in heavy black and limits of stele indicated by broken line; roots (labeled *a*, *b*, *c*, *d*, *e*) surrounded by continuous line; each figure shows stem stele, and most of them one or more roots, which lie within cortex of stem; cortex not shown except in fig. 22, after which roots have passed out to enter soil; *p*, primary root; *a*, youngest adventitious root; *e*, oldest root;  $\times 150$ .

FIG. 25.—Longitudinal section of stem;  $\times 775$ .

FIG. 26.—Longitudinal section of root;  $\times 775$ .

FIGS. 27-30.—Cross-sections of stems: condition in fig. 28 is generally found near apex, but this section was taken just above primary root, phloem (unshaded cells) is on radii alternating with xylem radii, and on a different circumference (this is not always true); fig. 29 shows section from same region in another plant; figs. 27-30,  $\times 775$ ; fig. 30,  $\times 375$ .

### PLATE X

FIGS. 31-37.—Figs. 31-33, cross-sections of roots; fig. 31, adult adventitious root from mature plant;  $\times 775$ ; fig. 33, adventitious root of sporeling after emerging from cortex;  $\times 375$ ; fig. 32, middle of primary root of sporeling;  $\times 775$ ; fig. 35, xylem connection at junction of adventitious root and stem xylem;  $\times 775$ ; figs. 34, 36, 37, three sporelings, one without primary root, a rare condition; fig. 36 shows method of cutting long and crooked specimens for sectioning;  $\times 5$ , with duplicates to right natural size.

# WATER-SOLUBILITY OF DRY MATTER IN RELATION TO CALCIUM NUTRITION OF NORMAL ORANGE AND LEMON LEAVES<sup>1</sup>

A. R. C. HAAS

## Introduction

It has been shown by KELLEY and CUMMINS (2), and later repeatedly confirmed by the writer, that normal mature leaves of trees of lemon and Washington Navel and Valencia orange differ very little in percentage of ash, and that this ash contains approximately 30-35 per cent of calcium. A study, therefore, of the narrow range of the sodium, potassium, calcium, and magnesium percentages in the ash of normal mature citrus leaves gives us very little opportunity to gain an understanding of differences in the behavior of the trees when subjected to the same external conditions. Numerous ash analyses of normal mature leaves of lemon and Navel and Valencia orange trees have failed to show any fundamental differences in the nutrition of these leaves.

Water-solubility studies conducted by REED and HAAS (3), upon the dry matter of normal Valencia orange trees grown in soil, have shown that approximately one-half of the calcium present is water-soluble, the ash of the water-soluble fraction being approximately 57 per cent. The water-solubility studies were restricted to Valencia orange trees, because it was believed that the analyses would show no important changes if they were also conducted upon any of the other varieties of citrus. After several years of soil, sand, and water-culture studies, made chiefly upon Valencia orange trees, the writer became concerned regarding the uniformity of the ash analyses of the various citrus varieties in relation to the diversity of the behavior of the trees. Had the ash analyses of the citrus varieties been somewhat different from one another, the present study would have suggested itself much earlier.

In this investigation it has been found that the ash of the water-

<sup>1</sup> Paper no. 175, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

insoluble fraction of the dry matter and the water-solubility of the calcium of normal mature lemon leaves are decidedly different from those of Navel and Valencia orange leaves, and that the age of the leaf determines in a large measure the water-solubility in a given variety.

### Investigation

The samples of leaves were collected from the trees in the experimental plots at the Rubidoux tract of the Citrus Experiment Station. The leaves were dried at 70–80° C. and finely ground. Samples of 10 gm. each were weighed into 150 cc. pyrex beakers, and the beakers then filled with distilled water and left to stand over night. The following morning the solutions were filtered and the leaf material transferred to the filter papers, where they were leached with successive small amounts of distilled water until a volume of about 500 cc. of filtrate was obtained. The filter paper and its contents were dried and ashed by igniting in a weighed dish at low heat, leaching out the soluble salts, drying, and reigniting the filter paper until free from carbon. The added soluble-salt solution was then evaporated and gently ignited to constant weight. The filtrate was evaporated nearly to dryness, transferred to a weighed dish on a hot plate, and when near dryness was placed in an electric oven at 100° C. to complete the drying process. The dried mass was then ignited at low heat, the soluble salts leached out and reignited, and treated as previously described. A point of interest in this connection was the fact that the dried water-soluble fraction of immature lemon leaves puffed up enormously on being ignited.

The ash of the water-soluble and that of the water-insoluble fractions were dissolved in dilute nitric acid, and after being filtered the solutions were made up to a volume of 500 cc. Calcium and magnesium were determined in each case, and in some cases sodium and potassium were also determined. The total phosphorus extractable with distilled water from the dry matter was determined by means of oxidizing the organic matter with magnesium nitrate.

Table I gives data in regard to the ash of the water-soluble and water-insoluble fractions of the dry matter of lemon leaves of different ages, and the water-solubility of the bases. The ash of the water-soluble fraction varies within a narrow range, lying between 3.5 and

TABLE I  
WATER-SOLUBILITY OF DRY MATTER OF NORMAL LEMON LEAVES OF DIFFERENT AGES, EXPRESSED AS PERCENTAGE DRY MATTER

LOCALITY (RUBIDOUX TRACT)	AGE	NO. OF LEAVES	WEIGHT		H <sub>2</sub> O CONTENT AS PERCENTAGE OF FRESH WEIGHT	ASH OF		CALCIUM		MAGNESIUM		SOLUBLE AS PER- CENTAGE OF TOTAL		SOLUBLE AS PER- CENTAGE OF TOTAL		SOLUBLE AS PER- CENTAGE OF TOTAL		PHOSPHORUS	
			Fresh gm.	Dry gm.		Sol- uble frac- tion	Insol- uble frac- tion	Sol- uble	Insol- uble	Sol- uble	Insol- uble	Sol- uble	Insol- uble	Sol- uble	Insol- uble	Sol- uble	Insol- uble	Sol- uble	Insol- uble
Collected June 22, 1927																			
Plot B, Eureka on sweet orange stock	Young.....	268	76	10	75.00	4.91	2.32	0.463	0.788	37.01	0.210	0.058	70.06	84.09	98.14	.....	.....	.....	.....
	Full sized, thin.....	160	134	34	74.62	4.99	4.31	0.370	1.501	21.06	0.154	0.120	57.75	76.93	90.87	.....	.....	.....	.....
	Nearly mature.....	100	120	48	60.00	4.71	11.36	0.813	3.939	17.11	0.131	0.061	71.22	75.84	96.23	.....	.....	.....	.....
Plot U, Eureka on grape- fruit stock	Young.....	575	58	16	72.41	5.15	1.45	0.470	0.424	52.57	0.263	0.063	80.68	.....	.....	.....	.....	.....	.....
	Full sized, thin.....	141	107	30	71.60	3.53	3.03	0.271	1.238	17.05	0.170	0.123	58.86	.....	.....	.....	.....	.....	.....
	Mature.....	74	98	44	55.16	4.55	11.47	0.935	4.307	17.84	0.204	0.073	79.34	.....	.....	.....	.....	.....	.....
Collected April 25, 1927																			
Variety Block, Belair lemon on sour orange stock	Young.....	350	65	17	73.85	5.37	2.22	0.273	0.735	27.35	0.202	0.088	70.00	.....	.....	.....	.....	.....	.....
	Full sized, thin.....	200	145	33	77.24	4.72	3.43	0.222	1.089	16.93	0.139	0.142	49.46	.....	.....	.....	.....	.....	.....
	Mature.....	100	140	59	57.86	4.36	11.22	0.903	4.266	17.47	0.216	0.107	66.87	.....	.....	.....	.....	.....	.....

5.4 per cent of the dry matter, and appears to be slightly lower in mature than in very young leaves. The ash of the water-insoluble fraction increases with the age of the leaves. When the leaves are full sized but thin, the two fractions are more nearly equal in ash content than at any other time in the development of the leaves. As the leaves become mature, the ash of the water-insoluble fraction increases rapidly, while that of the water-soluble fraction remains practically unchanged. Table I shows that the very young leaves contain approximately the same percentage of water as do those that are full sized but thin, while there is a decrease as maturity is approached.

The water-insoluble calcium of the dry matter increases gradually at first and most rapidly as maturity is approached, but changes very little after maturity is reached. There is some reason to expect that a curve showing the water-insoluble calcium, plotted against the age of the leaves, would follow the usual course of growth curves, although this has not been carried out as yet on citrus. The water-soluble calcium of the dry matter decreases slightly as the leaves become full sized but thin, and then increases toward maturity of the leaves. Calculations from the data have shown the usual increase in percentage of total calcium in the total ash with increasing age of the leaves. In table I, however, the insoluble calcium increases at a more rapid rate than the soluble calcium, with the result that the soluble calcium, when represented as a percentage of the total calcium, shows a decrease with increasing age of the leaves.<sup>4</sup>

The soluble magnesium when represented as a percentage of the total magnesium is least when the leaves are full sized but thin. Calculations of the percentage of total magnesium, sodium, and potassium present in the total ash show the usual decrease with increasing age, as found by KELLEY and CUMMINS. The soluble sodium, as a percentage of the total sodium present, decreases with increasing age of the leaves. Over 90 per cent of the total potassium is water-soluble. The percentage of water-soluble phosphorus decreased with increasing age of the leaves, but the percentage of insoluble phosphorus remained practically the same at the different ages.

The results for normal Navel and Valencia orange leaves are

given in table II. In contrast, the results for lemon leaves (table I) take on added importance. The percentage of ash from the water-soluble fraction of mature Navel and Valencia leaves is much higher than that of mature lemon leaves, while the converse is true of the percentage of ash from the insoluble fraction. The water-insolubility of the inorganic constituents of the lemon leaves, when full sized but thin, is greater than for Navel or Valencia orange leaves of the same stage of development. The data given for mature leaves of both varieties are typical of numerous determinations.

The calcium of the mature orange leaves in table II is of special interest, in that about 46-49 per cent of the total calcium of the dry matter is water-soluble, quite in contrast with the value of 17 per cent for mature lemon leaves. The percentages of soluble sodium and phosphorus and insoluble phosphorus decrease with increasing age of the leaves. There is no sharp distinction between the results obtained for Navel and for Valencia orange leaves. It is of additional interest to find, by calculation from the data given, that the soluble calcium as a percentage of the ash of the soluble fraction varies inversely with the values obtained for potassium.

The results presented emphasize the fact that ash analyses of normal citrus leaves, when supplemented with water-solubility data, may give us a dynamic conception of the inorganic constituents instead of a static one. The large water-solubility of the potassium in citrus leaves and the different degrees of solubility of the calcium may throw new light on the mottle-leaf problem.

The function of the large amount of soluble salts in mature Navel and Valencia orange leaves, in contrast with the lower amount in mature lemon leaves, may be of significance during severe desiccating winds, especially during the night time. It has been shown by HAAS and REED (1) that the increased calcium in Valencia orange leaves subject to desiccating winds is represented almost entirely by water-soluble calcium. It is of added interest now to determine what lemon leaves would do with an increased supply of calcium, in view of their normal maintenance of a low solubility for calcium. The use the various citrus leaves make of an insufficient supply of calcium becomes of great importance. The large differences between the soluble calcium of normal mature lemon and orange leaves may

TABLE II  
WATER-SOLUBILITY OF DRY MATTER OF NORMAL NAVEL AND VALENCIA ORANGE LEAVES OF DIFFERENT AGES,  
EXPRESSED AS PERCENTAGES DRY MATTER

LOCALITY (RUBIDOUX TRACT)	AGE	No. OF LEAVES	WEIGHT		H <sub>2</sub> O CONTENT AS PERCENTAGE FRESH WEIGHT	ASH OF		CALCIUM		SOLUBLE AS PER- CENTAGE OF TOTAL		MAGNESIUM		SOLUBLE AS PER- CENTAGE OF TOTAL		SOLUBLE AS PER- CENTAGE OF TOTAL SODIUM		PHOSPHORUS	
			Fresh gm.	Dry gm.		Sol- uble frac- tion	Insol- uble frac- tion	Sol- uble	Insol- uble	Sol- uble AS PER- CENTAGE OF TOTAL	Insol- uble	Sol- uble	Insol- uble	Sol- uble AS PER- CENTAGE OF TOTAL	Insol- uble	Sol- uble AS PER- CENTAGE OF TOTAL	Insol- uble	Sol- uble	Insol- uble
Collected June 22, 1927																			
Plot B, Navel	Young.....	326	60	15	75.00	5.18	2.02	0.553	0.614	47.38	0.216	0.084	0.084	72.00	85.44	08.03	0.351	0.069	
	Full sized, thin..	188	97	24	75.26	4.55	2.60	0.384	0.870	30.62	0.103	0.098	0.098	66.32	83.07	07.86	0.283	0.045	
	Mature.....	100	92	40	56.52	7.84	7.10	2.318	2.664	46.53	0.206	0.051	0.051	80.16	82.45	97.19	0.098	0.033	
Plot B, Valencia	Young.....	300	42	12	71.43	5.60	2.17	0.904	0.672	57.36	0.180	0.073	0.073	71.14	81.67	94.63	.....	.....	
	Full sized, thin..	115	76	21	72.37	5.39	2.98	0.876	1.106	48.26	0.102	0.042	0.042	70.83	77.09	97.59	.....	.....	
	Mature.....	100	90	37	58.89	7.66	7.28	2.595	2.626	48.82	0.196	0.056	0.056	77.78	68.28	94.39	.....	.....	



be a factor in the difference of behavior between the two species as regards sensitivity to toxic agents such as boron, to desiccating winds, and to hardness. The writer is continuing these most promising lines of investigation.

### Summary

1. The ash of normal citrus leaves of the same age shows practically no difference in composition for the citrus varieties examined. When the dry matter of these leaves is extracted with water, the ash and the calcium of the soluble fraction of mature normal Washington Navel and Valencia orange leaves are greater than those of Eureka lemon.

2. The water content of these leaves as a percentage of their fresh weight changes very little until after the leaves have reached their full size and have begun to mature.

3. The percentage of the total magnesium that is water-soluble is usually at a minimum at the time when the leaves have reached their full size and are still thin.

4. The percentage of the total sodium soluble in water decreases with increasing age of the leaves. Over 90 per cent of the potassium of the dry matter of citrus leaves is water-soluble. The water-soluble phosphorus of the dry matter decreases with increasing age of the leaves.

5. The relationship between the calcium nutrition of these citrus varieties and certain physiological characteristics is discussed to show the bearing the present paper has on the future direction of the investigation of citrus nutrition.

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# OVERCOMING DELAYED GERMINATION OF *NELUMBO LUTEA*

JAMES A. JONES

## Introduction

In this investigation it was sought to determine the factors involved in the delayed germination of *Nelumbo lutea*. Seeds were obtained from the Terrell Aquatic Farm of Oshkosh, Wisconsin, for the purpose of determining a method of overcoming the delayed germination, and thereby enabling the dealer to guarantee a high percentage of germination to those growing this plant for water gardens and game preserves. OHGA<sup>1</sup> has reported the results of investigations with *Nelumbo nucifera*. Since the coats of the fruits were found to be impervious to water, the fruits were prepared for germination by treating for five hours with concentrated sulphuric acid, followed by washing and drying.

## Method

Control seeds were placed in water at a temperature of 15°–20° C. After eighteen months no visible change had been observed, evidently owing to the extremely hard coat typical of the *Nelumbo* seeds. When the seed coats were filed to expose the brown nucellus tissue before soaking in water, without exception they began immediately to absorb water and became swollen. Seeds having their coats broken with a vise and by abrasion with an emery wheel showed similar results when the embryo was not injured.

Since these tests indicated that the cause of delay in germination was the seed coat, seeds were treated by immersing in concentrated sulphuric acid and removed in groups of one hundred seeds, at intervals of one hour, extending to seventy-two hours. Fifty of the seeds in each case were quickly and thoroughly washed in tap water, to prevent heating by the addition of water to the acid-soaked coat; the other fifty seeds in each test were washed in dilute NH<sub>4</sub>OH.

<sup>1</sup> OHGA, I., On the longevity of seeds of *Nelumbo nucifera*. Bot. Mag. Tokyo 37:439–444. 1923.

The seeds were then submerged in water in glass jars, at room temperature, and the water changed frequently at first, to prevent it becoming acid from the acid still left in the thick seed covering. Other seeds similarly treated were dried at room temperature and stored for a period of fourteen days before they were allowed to germinate.

Tests were made to determine the effect of low temperature upon the time of germination. Seeds were allowed to remain in air at a temperature of  $0^{\circ}$ – $5.5^{\circ}$  C. for twenty-four hours, and others were placed in water and kept at the same temperature for an identical period. These tests were also run for a period of forty-eight and seventy-two hours, after which the seeds were placed in water in glass jars to germinate.

Seeds were allowed to soak in ethyl alcohol and removed at intervals of thirty minutes and the test terminated at six hours. These seeds were washed in tap water as soon as they were removed, and placed in glass jars to germinate.

### Results

Seeds when treated with concentrated sulphuric acid for varying lengths of time up to seventy-two hours, and then washed with tap water, showed that the treatment for one hour was of insufficient duration to produce result. Other tests showed 100 per cent germination in nearly every instance. When germination was not complete the cause apparently was defective seeds which showed no signs of life upon examination. The most practical results were produced by treatment for five hours, showing 10 per cent of the seeds germinating in four days and 100 per cent germinating in thirty-four days. Dilute  $\text{NH}_4\text{OH}$  and tap water were equally effective as neutralizing agents. Seeds similarly treated but dried and stored for fourteen days produced parallel germination results. Untreated seeds which were in water for fourteen months and showed no signs of water absorption or germination responded quickly to this treatment. In one instance complete germination resulted in seven days.

The seeds which had been exposed to low temperature, in both the dry state and in water, showed no seeds swelling or germinating after remaining in water in glass jars for fourteen months.

No germination results were produced from seeds treated with

ethyl alcohol and washed with tap water, although they were kept in correct germinating condition for eighteen months. VERSCHAFFELT<sup>2</sup> has found that water fails to enter the seeds of legumes because it will not wet the walls of the micropyles, but if the seed is first soaked for an hour in ethyl alcohol, it readily enters the openings and furnishes a path for the inward diffusion of water.

### Discussion

Although the surface of the testa of the seeds of *Nelumbo lutea* contains many small pores, in the outer walls of the seeds a part of the cellulose has been converted into suberin, which is waterproof and brownish in color. EWART<sup>3</sup> has found that in some hard seeds all layers of the integument are highly impervious to water, and GUPPY<sup>4</sup> claims that this is prevalent among hard-coated forms with large seeds.

It has been shown that the embryos are not dormant, and that germination can be produced by mechanical weakening of the seed coat.

### Summary

1. In spite of the presence of a large number of small pores in the seed coat, the seeds of *Nelumbo lutea* did not absorb water and germinate, even after eighteen months' soaking at room temperature.
2. When the seed coats were broken without injuring the embryos, the seeds germinated without exception.
3. Seeds may be prepared for germination by treatment for five hours with concentrated sulphuric acid, following by thorough washing in tap water and later by drying on a screen to eliminate immediate germination. Seeds so treated may be stored by the commercial grower and shipped dry as ordered.

This work has been done under the direction of Dr. J. F. GROVES of Ripon College.

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<sup>2</sup> VERSCHAFFELT, E., Rec. Trav. Bot. Neerland 9:401. 1912.

<sup>3</sup> EWART, A. J., On the longevity of seeds. pp. 210. Victoria, Australia. 1908.

<sup>4</sup> GUPPY, H. B., Studies in seeds and fruits. pp. 585. London. 1912.

## STIMULATING EFFECT OF AMINO ACIDS ON SUGAR METABOLISM OF PLANT AND ANIMAL CELLS

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AND MAUDE WILLIAMS

(WITH ONE FIGURE)

LAVOISIER (1) found that the ingestion of food increased oxidation or metabolism in the animal. RUBNER (3) showed that meat or protein increased metabolism more than did fat or carbohydrate. LUSK (2) found that the amino acids increased metabolism or heat production, and since protein is presented to the tissues of animals as amino acids, the stimulating effect of protein is in reality due to the amino acids. SPOEHR and MCGEE showed that glycine increased sugar utilization by *Helianthus* (4).

The object of this investigation was to determine the effect of optically active and optically inactive amino acids on the sugar metabolism of the plant cell, *Spirogyra porticalis*, and the animal cell, *Paramecium caudatum*. The spirogyra grew in large quantities and fairly pure cultures in a near-by lake. The paramecia were grown in large numbers on an infusion made of lake water and alfalfa. They were collected and washed free of débris with the use of a small centrifugalizing machine. The centrifugalizing tubes were graduated in centimeters, so that the paramecia were measured as they were collected. Air was kept continuously bubbling through the liquid containing the paramecia to insure an adequate supply of oxygen. Dextrose, levulose, and galactose were the sugars used. Sugar determinations were made according to the method of BENEDICT. The optically active amino acids used were l-leucine, l-tyrosine, l-aspartic acid, l-histidine, d-glutamic acid, l-tryptophan, aminoids; the optically inactive ones were dl-alanine, dl-isoleucine, glycine, dl-phenylalanine, dl-norleucine, dl-leucine, and dl-valine.

The following is the description of a typical experiment. Seventy-five cc. of paramecia was collected, washed, and measured as described, and then introduced into 1500 cc. of aerated lake water, to

which was added 1500 mg. of dextrose. After the sugar was dissolved, this 1500 cc. of paramecia-sugar preparation, while being thoroughly mixed by pouring from one vessel to another, was separated into 100 cc. batches. Each 100 cc. portion was introduced into a 200 cc. sedimentation glass, and air was bubbled through it to insure an adequate supply of oxygen to the paramecia. One hundred mg. of each of the amino acids was introduced into beakers and 10 cc. water added and heated. A sufficient quantity of a saturated solution of sodium bicarbonate was added to form the sodium salts of the acids. Each of these 10 cc. solutions was added to 100 cc. of sugar-paramecia preparation, and 10 cc. of water was added to the controls. Sugar determinations were made immediately and subsequently at intervals. Spirogyra sugar preparations were made as follows. A large batch of spirogyra was collected, brought to the laboratory, and the excess of water removed by gently squeezing with the hands. This large batch was then divided into batches of 40 gm. each. These were then introduced into 200 cc. of 0.1 per cent dextrose solutions in flat bottom dishes 16 cm. in diameter. The sodium salts of the different amino acids were prepared and added as in the paramecia-sugar preparation, and sugar determinations were made immediately and subsequently at intervals. The results of the average of five experiments for spirogyra and five for paramecia are shown in fig. 1. It will be seen that the optically inactive amino acids did not increase the sugar metabolism in either paramecium or spirogyra over the controls, whereas the optically active amino acids produced a great increase in sugar metabolism in both the spirogyra and the paramecium. It may be seen further that the aminoids increased sugar metabolism both in the paramecium and spirogyra. The aminoids were a commercially prepared meat digest containing the naturally occurring amino acids, biuret-free, with a total nitrogen of 10.93 per cent, and the amino nitrogen 75 per cent of the total nitrogen.

Experiments were carried out using levulose and galactose, similar to those in which dextrose was used, with fairly comparable results. Paramecium used all three of the sugars more rapidly than the spirogyra, in keeping with the fact that the metabolic rate of the plant is less intense than that of the animal. It was found also that

paramecium as well as spirogyra used dextrose and levulose more rapidly than galactose, just as is the case with the higher animals and man. It was also found that insulin increased the rate of utilization of these sugars, both by the spirogyra and the paramecium, again resembling the higher animals and man.

It is recognized that optical activity plays a rôle in the plant kingdom. Yeast, for example, will use the dextrorotatory form of

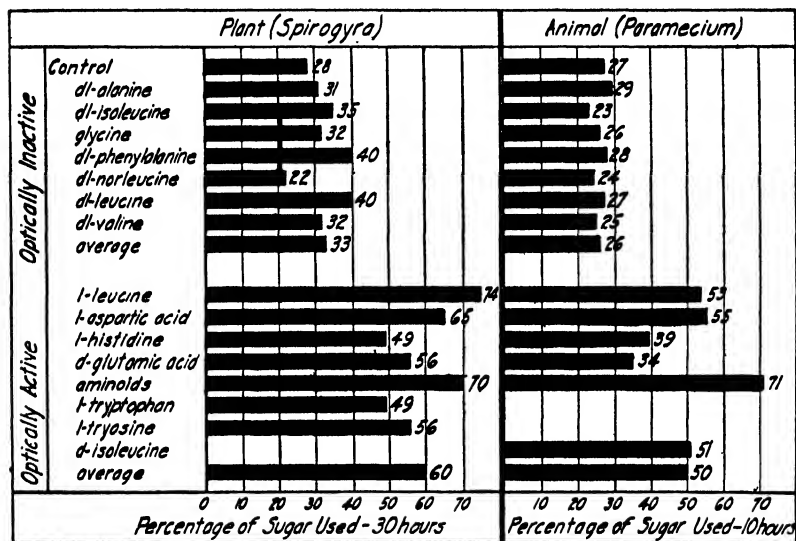


FIG. 1.—Chart showing that optically active amino acids increase rate of sugar utilization in spirogyra and paramecium, whereas optically inactive amino acids do not.

glucose but not the levorotatory form, so if yeast be added to a mixture of dextrorotatory and levorotatory glucose, it will use the dextrorotatory and leave the levorotatory. This is one method of separating these two forms of sugar. It is also known that practically all of the naturally occurring amino acids, and hence the ones normally used, are optically active. The observation reported in this paper, that only the optically active amino acids stimulate metabolism, would seem to attach significance to the fact that with one exception all the naturally occurring amino acids are optically active.

#### 1. The optically active amino acids stimulate sugar metabolism

### Summary

in the plant cell, spirogyra, and the animal cell, paramecium, while the optically inactive ones do not.

2. Paramecium uses sugar much more rapidly than spirogyra, in keeping with the more intense metabolism in animals than in plants.

3. Spirogyra and paramecium use dextrose and levulose more rapidly than galactose, just as is the case with higher animals and man.

4. Insulin increases the rate of utilization of the sugars in paramecium and spirogyra, similar to its action in the higher animals and man.

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# CURRENT LITERATURE

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## BOOK REVIEWS

### Notable books on algae

Three books on algae have recently appeared which will be of use to all interested in this group of plants.

WEST's volume<sup>1</sup> on British freshwater algae, which appeared in 1904, made a place for itself, not only in England but throughout the world; for algae are so cosmopolitan that the book was nearly as useful for ponds about Chicago as for ponds about London. The edition was exhausted even before the death of the author, who was collecting material, notes, and illustrations for a more extensive treatment of the group. At his untimely death the work was still far from the publication stage, but it probably represents much that he had in mind for the new work. This may be due, in considerable measure, to Dr. NELLIE CARTER, who for several years was associated with Professor WEST in his algological studies, and to whom FRITSCH makes acknowledgments for the discussion of many difficult points. Much of the most valuable part of the first edition has been preserved, especially the descriptions of genera and the notes on occurrence and distribution. Some material has been omitted, but much more has been added, so that the size and scope of the work have been increased.

The first 50 pages deal with biology and general features of life histories and development. While the rest of the book is arranged taxonomically and contains the taxonomic keys, it is full of biological notes and descriptions of life histories; so that, in its method of treatment, it lies between such a taxonomic account as we find in ENGLER's *Pflanzenfamilien* and a morphological biological account like that of OLTMANN'S. The directions for collecting algae, keeping them alive in the laboratory, and for making cultures will be useful.

While little attention is paid to alternation of generations, the remark is made that as far as one accepts the diploid and haploid condition as representing alternating generations, "most sexually reproducing freshwater algae may be said to show slight indications of antithetic alternation of generations." The reviewer believes that the algae show the origin of alternation of generations, and, therefore, the true nature of this phenomenon. Plants below the level of sexuality have only one generation, the  $x$ , or haploid. The diploid phase arose by the fusion of gametes, and, consequently, must be present not only in "most sexually reproducing freshwater algae," but in all plants and animals which have

<sup>1</sup> WEST, G. S., and FRITSCH, F. E., A treatise on the British freshwater algae. 8vo. pp. xvi+534. figs. 207. Cambridge University Press. 1927.

sexuality. If the zygote divides several times before reduction of chromosomes takes place, it builds up a more or less conspicuous diploid generation; but even when the reduction of chromosomes takes place during the first two divisions of the zygote, as in *Spirogyra* and all the rest of the Chlorophyceae which have been examined, the diploid generation has been started. If, in *Coleochaete*, the reduction should not be completed until the 16-cell stage has been reached, there would be a diploid body which would be recognized as comparable with that of *Riccia*. Logically, it makes no difference whether there are 50 mitoses, or whether there are 25, 10, or only the two which are necessary to bring the plant back to the original haploid condition. Much confusion has arisen from the assumption that haploid generation and gametophyte generation are synonymous terms, and that diploid generation means the same as sporophyte generation. From the liverworts to the orchids and in many thallophytes the terms are synonymous; but in many thallophytes, where alternation is originating and becoming established, the terms are not synonymous.

It has long been recognized that it is difficult to separate the algae from the pigmented Flagellates. OLTMANN began his book on the *Morphologie und Biologie der Algen* with the statement that the algae go back, phylogenetically, to the Flagellates; and this view has become a conviction with most students of algae. It was the fact that many Flagellates are just as distinctly animals as others are distinctly plants that led some zoologists to regard the Volvocales as animals because they are obviously related to the Flagellates. The present volume, by including the pigmented Flagellates in a textbook treatise on algae, will bring this group to the attention of a large number of teachers who are not specialists in algae, and thus lead to a better understanding of the Volvocales.

Morphologists and cytologists will readily agree that the diatoms should not be associated with the Phaeophyceae; but they will not so readily agree that they should be removed from their association with the desmids and the rest of that group. I should not agree that the evidence for placing the diatoms in the Zygomycetes is superficial; it is strictly internal.

The Cyanophyceae are placed at the end of the book. If they are to be included in the algae at all, their simple nuclear structure and the fact that not a single one of them has reached the level of sexuality might give them a place even lower than the Flagellates.

The artificial keys and the arrangement of material give the book a taxonomic aspect, and make it easy to find data in regard to any particular form; but, besides the descriptions of families, genera, and species, there are special accounts of distribution, structure, development, and the biology of life histories, in addition to the general accounts given in the first 50 pages.

Unless the present edition is much larger than the previous one, it will soon be exhausted. There is need for a similar account in English, including the algae of the world, both freshwater and marine.

PASCHER's<sup>2</sup> immense work on the freshwater flora of Germany, Austria, and Switzerland is progressing. The volume on the Cyanophyceae, by GEITLER, has already been noted in this journal.<sup>3</sup> The present work, Volume IV, by PASCHER himself, is devoted entirely to the Volvocales, with the exception of a few pages of general introduction to the Chlorophyceae. It is the first comprehensive treatment of this important order.

The book should be regarded as a taxonomic monograph of the Volvocales, but it is more than an assemblage of taxonomic keys. The descriptions are very thorough, with discussions of relationships and some attention to biological conditions; but the taxonomic feature is dominant, just as the biological and morphological features are dominant in the work of WEST and FRITSCH. The synonymy is everywhere very complete. *Chlamydomonas* may be taken as an example. More than three pages are devoted to the synonymy and diagnosis of the genus, and 132 pages are devoted to keys and detailed descriptions of the 146 recognized species, and three pages more to doubtful species. Illustrations of critical features add to the value of the diagnoses.

It is fortunate that one so thoroughly acquainted with the Volvocales has devoted so much space to a description of the more difficult lower members of the order. This treatment will enable those who are not specialists to understand something of the relationships of the Volvocales to the Flagellates and to other groups.

A volume by MELCHIOR<sup>4</sup> on the algae covers the Cyanophyceae, Flagellatae, Dinoflagellatae, and Bacillariales. It is the first volume of a series presenting all the algae, and is the second and much enlarged edition of the book by LINDAU, who outlined a complete series dealing with all the Cryptogams, under the title *Kryptogamenflora für Anfänger*. While the treatment is not so detailed as in PASCHER's work, it would look rather difficult for a beginner. After about 40 pages of introduction, the rest of the book is strictly taxonomic, with keys in German, as in the PASCHER series.

The fact that these three major books have appeared within a year indicates an interest in the lower Cryptogams.—C. J. CHAMBERLAIN.

#### An introductory botany

A textbook by TORREY<sup>5</sup> dealing with introductory botany deserves the consideration of teachers, since the author has definite and pronounced views in regard to the teaching of this subject. He is clearly of the opinion that a serious

<sup>2</sup> PASCHER, A., Die Süßwasser-Flora Deutschlands, Oesterreichs und der Schweiz. Heft 4: Volvocales. 8vo. pp. vi+506. figs. 451. Jena: Gustav Fischer. 1927.

<sup>3</sup> BOT. GAZ. 82:341. 1926.

<sup>4</sup> MELCHIOR, HANS, Die Algen, Erste Ableitung. 8vo. pp. viii+314. figs. 489. Berlin: Julius Springer. 1926.

<sup>5</sup> TORREY, R. E., Introductory botany, 2d ed. Vol. I: Structure and classification of seed plants. pp. xvii+141. figs. 77; Vol. II: The anatomy and physiology of seed plants. pp. viii+127. figs. 71. Mass. Agric. Coll. Book Store. 1926. \$1.65 and \$1.35.

effort should be made to reconcile the purely inductive and the philosophical points of view in the teaching of botanical science to beginners. There can be no doubt that he is largely right in this attitude, because the results of the purely inductive method are certainly discouraging from the standpoint of enlightened pedagogy. The book under discussion makes a happy compromise between the narrowness of the mere observationist and the too comprehensive superficiality of the purely philosophical mind.

The author intersperses his descriptions of the various aspects of the subject which he treats with pregnant and forceful phrases, which should serve as vigorous mental stimulants to the classes who are fortunate enough to make use of this new textbook. Many of the illustrations are original, and show not only good technique but admirable judgment in the matter of utility. Since the author adopts a general point of view, he is always at pains to connect his facts with an interesting train of reasoning. In this connection it may be remarked that the present work is the only one which embodies recent results in the evolution of the various elements and organs of woody plants. The treatment of the origin of the herbaceous dicotyledonous and monocotyledonous types is particularly happy, and illustrated by original drawings which serve to illuminate an important subject particularly difficult to the beginner. The author makes very clear the importance of the herbaceous type in connection with the sustenance of the human species. The subjects of taxonomy, paleobotany, plant physiology, ecology, evolution, etc., are also handled in an illuminating fashion and with excellent judgment, and in such a way as to inspire interest in students whose mental processes are in any way active.

The publication of this excellent work in two small volumes under State auspices has in all probability stood in the way of its adoption by a wider range of colleges. The author, however, has made a distinct contribution to biological pedagogy in the way of a new method of teaching essential structures. One of the bugbears of biology has been the making of endless drawings in the laboratory. This has militated against the popularity of the subject with the beginner, even at a time when biology has again entered the position of controversial interest which it occupied so brilliantly two generations ago. TORREY has attempted to meet this difficulty by providing line reproductions of drawings, on a sufficient scale of enlargement for the student to recognize and label the parts, whether gross and superficial or minute and microscopical, accurately and with comprehension. This is a distinct advance pedagogically, and seems to present advantages from the standpoint of elementary instruction. It may be added that TORREY's method has been tried out with success in the botanical portion of a cultural course in Harvard University. It is to be noted that the same method of treatment has been more recently adopted in zoology. In conclusion, one may remark that in this book we have a concise, interesting, well planned, and decidedly novel presentation of the subject, which will probably meet the needs of many teachers who are dissatisfied with the books at present available for elementary instruction in botany.—E. C. JEFFREY.

## NOTES FOR STUDENTS

**Accuracy of measuring concentration of hydrogen ions.**—Since 1920, when BILMANN described his quinhydrone electrode, this method has been more and more adopted in measuring the hydrogen ions in soils. The hydrogen electrode is now but little used in such researches, owing to the length of time and trouble involved in the measurements. The quinhydrone method and the colorimetric method are now chiefly employed. As regard the accuracy of the two methods, there seems to be a difference of opinion.

With a view to ascertaining the degree of accuracy attainable in measuring the hydrogen ion concentration of soils, OLSEN and LINDERSTRM-LANG<sup>6</sup> made a thorough investigation of the different methods.

Measurement with the hydrogen electrode has been regarded as a standard method, with which values found by the other two methods may be compared. However, the ordinary hydrogen electrode values cannot be used for comparison where the pH value of the soil exceeds about 5.0, as the carbonic acid is gradually given off in the course of measurement and the pH value is altered.

From investigations including measurements of 100 soil samples the quinhydrone electrode gave, in some cases, a value 0.8 too high, and it is impossible to introduce any correction for this. In the colorimetric method a correction can be introduced, and when this is done the pH values of soil samples can be measured with an accuracy of  $\pm 0.15$  pH. In general, soils cannot be measured more accurately than this. In the great majority of cases this will be quite sufficient in soil investigations, as the organisms of the soil are not appreciably sensitive to small differences in pH.—J. ISENBARGER.

**An illustrated flora of China.**—HU and CHUN<sup>7</sup> of the National Southeastern University at Nanking, China, have published the first fascicle of a remarkably elaborate presentation of the plants of China. The large size ( $48 \times 31$  cm.) makes the handsome plates unusually impressive. This first fascicle is dedicated to CHARLES S. SARGENT, in memory of his interest in the woody flora of China. Fifty species are described, in 39 genera, in both English and Chinese, with the facts of distribution and a general account of habitats and uses. Most of them are woody plants, the largest group being the Coniferae. The plates are so large and well prepared that they seem almost as effective as herbarium specimens.—J. M. C.

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<sup>6</sup> OLSEN, C., and LINDERSTRM-LANG, K., On the accuracy of the various methods of measuring concentration of hydrogen ions in soil. *Compt. Rend. Travaux Laboratoire Carlsberg*. 17: no. 1. 1927.

<sup>7</sup> HU, HSEN-HSU, and CHUN, WOOD-YOUNG, *Icones Plantarum Sinicarum*. Fascicle I. pp. 50. pls. 50. 1927.

# THE BOTANICAL GAZETTE

*June 1928*

## STUDIES OF PLANTS IN THE BLACK HILLS OF SOUTH DAKOTA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 380

HERMAN E. HAYWARD

(WITH FIFTEEN FIGURES)

### **Purpose and scope of study**

The Black Hills of South Dakota have for many years extended an attraction because of their unique geographical position, suggesting as it does hidden possibilities of interesting floral situations and associational relationships. The present study has grown out of an increasing desire to investigate the situation at close range, and to study the flora from the floristic and successional points of view.

The region was first visited during the summer of 1926, and six weeks were devoted to a preliminary reconnaissance. This resulted in a decision to attack the problem from two angles, floristic and formational. The intermixture of northern, southern, western, and eastern elements in the flora seemed of sufficient interest to justify a floristic analysis. The results of this phase of the survey constitute part I of this paper. The study dealing with the plant formations was carried on during the summer of 1927, the field work extending from June 8 to August 15. During this time studies were made of habitats that had been selected as a result of the field work of 1926 (fig. 1). Part II embodies an analysis of the plant formations thus determined.

### **Geography and topography**

The Black Hills are located in southwestern South Dakota and eastern Wyoming, extending from latitude  $43^{\circ} 15'$  to  $44^{\circ} 30' N.$ , and

from longitude  $103^{\circ} 10'$  to  $104^{\circ} 20'$  W. (fig. 2). The length of the higher portion of the uplift is about 100 miles and it is 50 miles across at its greatest width. Of a total area of about 4500 square

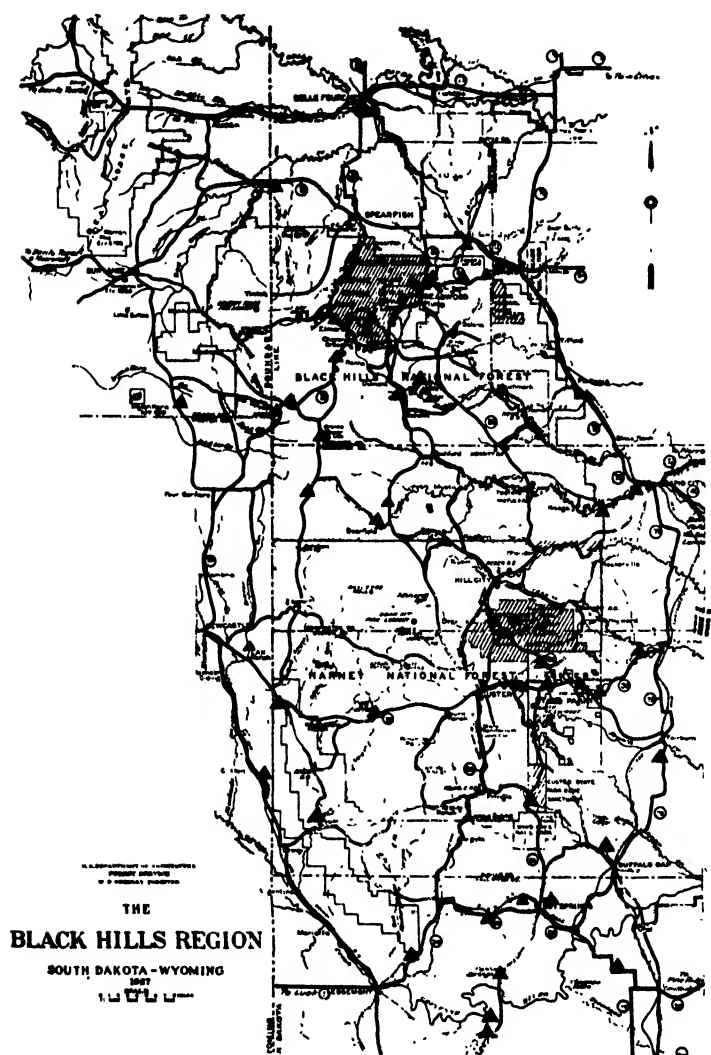


FIG. 1.—Black Hills region; black triangles indicate points at which studies were made.

miles, approximately two-thirds are in South Dakota, while one-third is in Wyoming.

The Black Hills rise several thousand feet above the adjacent plains, whose elevations range from 2500 feet near Belle Fourche and 3000 feet near the Cheyenne River, to 3300 or 3500 feet at the

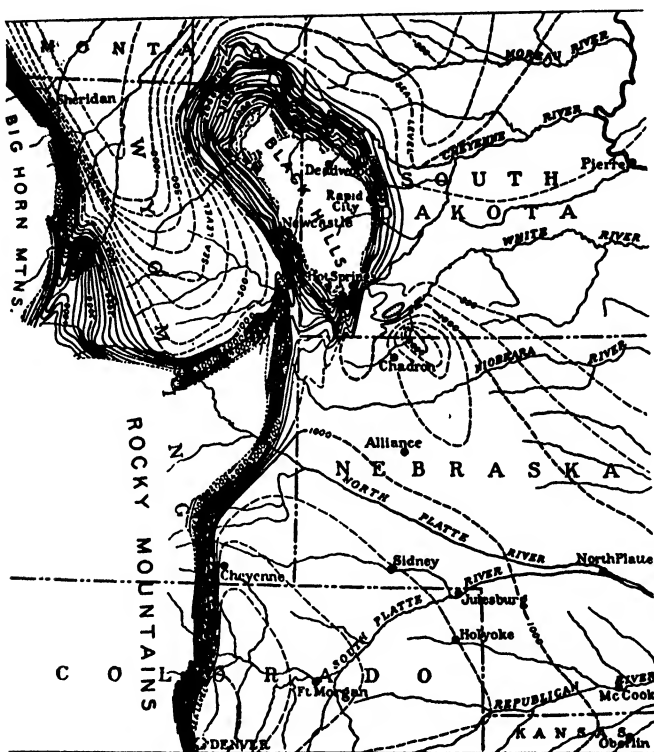


FIG. 2.—Black Hills, showing their geographical position

outer edge of the Hogback Ridge. The highest point in the Hills is Harney Peak, with an elevation of 7242 feet.

The topography (fig. 3) has been so clearly described by DARTON (8) that it can best be presented by giving a condensed summary of his account. The four outstanding features are the Hogback Ridge, the Red Valley, the Limestone Plateau, and the central area.

The Hogback Ridge forms the outer rim of the Hills, completely



encircling them. For the most part it consists of a single ridge of hard sandstone, which presents a steep face toward the Red Valley, and rises abruptly several hundred feet above the bottom of the valley, to an elevation of 3800 feet near Rapid City, and 4900 feet



FIG. 3.—Topographic map of the Black Hills showing Hogback Ridge, Red Valley, Limestone Plateau, and central hills.

at Elk Mountain. The outer side slopes gradually down to the plains. It is cut at numerous places by gaps, through which the streams arising in the hills pass out to join the waters of the Cheyenne and Belle Fourche rivers.

The Red Valley extends almost continuously around the Black Hills, and is one of the most striking features of the region because of the red color of its soil and the absence of trees. Its outer side is

the steep inner face of the Hogback Ridge previously mentioned, while its inner side consists of fairly steep limestone slopes. It does not exceed 2 miles in width, and in some places is much narrower, due to the steep dip of the strata. The altitude of the valley along the east side is 3300-3500 feet.

The Limestone Plateau is an interior highland belt within the Red Valley, ranging 2-15 miles in width. The western portion, which is broader than the eastern, slopes gently downward to the Red



FIG. 4.—Rim of Limestone Plateau as seen from east near Castle Creek

Valley, but its eastern or inner side is lined by abrupt cliffs that rise in places 800 feet above the central valleys (fig. 4). The plateau is higher than a large portion of the central area, attaining an altitude of 7100 feet at Crooks Tower, and thus forming the main divide of the Black Hills. South of the headwaters of Beaver Creek the plateau swings toward the eastern side of the Hills, where it is much narrower than on the western side, forming a ridge with a steep western face that reaches altitudes ranging from 5000 to 6600 feet. Streams arising at the higher altitudes of the western plateau have cut deep canyons in the limestone, and, after crossing the central region of crystalline rocks, have cut their way through the eastern ridge and joined the Cheyenne River.

The central area includes scattered rocky ridges and mountain peaks lying within the encircling limestone area. Most of these range from 5000 to 6600 feet in height, although several peaks are approximately 7000 feet, and the granite crags of Harney Peak exceed that figure (fig. 5). Throughout this area are parklike valleys, the wider ones being at the heads of canyons that extend outward to the northeast, east, and south.



FIG. 5.—The Needles in central hills near Harney Peak, habitat of *Pinus flexilis*

### Drainage

The Black Hills lie between the Belle Fourche and the Cheyenne rivers. The former, running northeastward from its source in Wyoming, makes an abrupt turn near the Montana-Wyoming line north of the Bear Lodge Mountains, flows in a southeasterly direction to Belle Fourche, and ultimately joins the Cheyenne about 50 miles east of Bear Butte (fig. 6). The Cheyenne River skirts the southern foothills, swings northeastward to its junction with the Belle Fourche, and finally empties into the Missouri River. From the watershed of the Limestone Plateau, Red Water and Inyankara creeks flow northwest into the Belle Fourche River; while Beaver Creek flows south, joining the Cheyenne at Edgemont. The eastern watershed is more plentifully supplied with streams. Spearfish and Whitewood creeks flow northeastward to join the Belle Fourche.

Elk, Boxelder, Rapid, Spring, and French creeks, together with Fall River, flow into the Cheyenne.

The erosive action of these streams has resulted in deep canyons whose sheer walls rise many hundreds of feet. The most notable of these is Spearfish Canyon, which is more than 1000 feet deep. It is in these northern canyons that the greatest degree of mesophytism is found, many of them being rich in moisture-requiring ferns and herbs.



FIG. 6.—Bear Butte: one of the Tertiary igneous intrusions characteristic of the northern hills.

### Climate

The climate<sup>1</sup> is characterized by late spring rains, summers that are hot and relatively dry, and cold winters with a moderate snowfall. The mean annual temperature for five stations, Deadwood, Fort Meade, Hermosa, Rapid City, and Spearfish is 45.6° F., while the midsummer average for the same stations is 70°. There is a great daily range of temperature in the summer, the days being

<sup>1</sup> The figures given in the section on climate were compiled from summaries of climatological data for the United States for sections 25 and 33 up to 1920 inclusive, and from annual and monthly reports from that date up to September 1, 1927. See also DARTON (8).

very hot at midday and the nights invariably cool. Winter temperatures for the same stations in January average from  $22^{\circ}$  to  $25^{\circ}$ .

In the central hills at the higher elevations, frosts begin early in the fall and continue until very late in the spring. At Deadwood the average length of the growing season is only 107 days, from May 31 to September 15, and it is not uncommon for frosts to occur as late as the middle of June and as early as August. In the eastern foothills the growing season is considerably longer than in the western, three stations in the former averaging 141 days, as compared with an average of 113 days for the same number of stations in the latter.

The most striking feature of the climate is the extreme variability of the precipitation from year to year at a given station, and from place to place in a given year. Thus Rapid City had 25.10 inches in 1923 and 14.91 inches the following year, and Lead had 16.49 inches in 1921 and 35.36 inches in 1922. The average annual precipitation in 1926 for fourteen stations within the Black Hills region was 20.97 inches; but the range of precipitation at the several stations varied from 14.67 inches at Belle Fourche to 38.26 inches at Harvey's Ranch in the central hills.

On the basis of complete figures for twenty-one stations, May and June are the months of greatest precipitation, the latter slightly exceeding the former. The combined rainfall of the two months is 30 per cent of the total annual figure. Nearly 60 per cent of the precipitation occurs during the growing season, from May to September inclusive.

The northern hills have the greatest rainfall. Five stations give an average of 24.45 inches, as compared with 21.34 inches at four stations in the central hills. The foothills are much drier, the average annual rainfall for ten stations being 18.25 inches. The Black Hills receive appreciably more moisture than the surrounding plains. Figures for South Dakota, west of the Missouri River and exclusive of the stations in the Black Hills, show an average annual precipitation of 17.21 inches; and northeastern Wyoming east of the Big Horn Mountains has only 15.73 inches.

It is evident from the foregoing that precipitation is one of the most important factors in the interpretation of the plant life, since

there are so many significant variations in the rainfall. Total precipitation in the Black Hills as a unit is of no value in studies of plants in this area, and it is necessary to consider each habitat separately in dealing with this factor.

### Geologic history

Probably no other region in the United States has received more careful attention from the geologist than the Black Hills. The reason for this is twofold. From the purely scientific standpoint, they afford one of the most complete and understandable pictures of past geologic processes to be found in western United States. The situation is almost diagrammatic in its major details. As WARD (30) states: "The Black Hills are an object lesson in geology." On the other hand, the discovery of gold in the hills led to a much more careful study of this region than is accorded to one lacking in valuable mineral deposits. It is not necessary, therefore, to do more than indicate briefly the record of major geologic events.<sup>2</sup>

The story of the making of the Black Hills is one of alternating epochs of submergence with accompanying deposition, followed by periods of uplift with resulting erosion and planation. Thus in pre-Cambrian times, the record indicates deposition of sedimentary materials on the floor of a sea, followed by a period of igneous activity which resulted in the granite intrusions that now form the central granite core of the Harney region. During the period of Cambrian submergence it is probable that the Black Hills, like the Big Horn Mountains to the west, formed an island in this great inland sea. The record of Ordovician-Devonian time is extremely meager, being represented only by the Upper Ordovician Whitewood limestone, which is found in the northwestern portion of the Black Hills. In the Carboniferous there was again a period of submergence that extended over the greater portion of the Rocky Mountain province. The early Mississippian seas laid down calcareous sediments, and in later Carboniferous (Pennsylvanian) time much fine sand was deposited. Then followed uplift, and the accumulation of

<sup>2</sup> For a bibliography of the geologic work done in the Black Hills, see O'HARRA, C. C., South Dakota School of Mines, Bull. 11. 1917; also DARTON, N. H., and PAIGE, SYDNEY, U. S. Geol. Sur., Folio no. 219. 1925.

the lower red beds of the Opeche formation of probable Permian time.

Widespread submergence is again indicated by the deposition of the thin Minnekahta formation by sea water, and then came a continuance of the red bed deposition which was laid down by shallow salty lakes. Extensive uplift took place in Triassic and Jurassic time, followed by cycles of submergence and uplift in Cretaceous time, with the accompanying deposition of various sandstones and shales. In Tertiary time the Black Hills dome was uplifted to a considerable height; and, by the Oligocene epoch, the principal topography as now existent was established. In connection with this general uplift, it is probable that the igneous intrusions that are so common in the northern hills took place (fig. 6).

Continued mountain growth occurred after the Oligocene, and this was followed by extensive erosion. If there were deposits of Miocene and Pliocene formations, they must have been removed by subsequent erosion, as no representatives of these epochs have been found in the region. The Quaternary shows evidence of some additional uplift, as well as widespread erosion which removed many of the preceding deposits.

### Glaciation

There is no evidence of glaciation in the Black Hills, and it is probable that plant life continued to exist there during glacial time. The glacial epoch must have affected the climatic conditions of the region profoundly, however, and it is therefore pertinent to note the proximity of the glacial area of the Wisconsin age.

According to UPHAM (26), the Dakota lobe of the Wisconsin ice sheet "from its junction with the Minnesota lobe near the head of the Coteau des Prairies, 25 miles west of Lake Traverse and Brown's Valley, at first reached about 200 miles south along the valley of the James or Dakota River to Yankton and the Missouri." Its western margin approximated the present course of the Missouri River, swinging northward across the state of South Dakota into central North Dakota, and thence westward across northern Montana. The edge of the glacial ice sheet at its greatest extent, therefore, was approximately 150 miles from the Black Hills.

There is evidence of local glaciation in the Big Horn Mountains.

which lie 100 miles west of the Hills. SALISBURY (23) states: "the Big Horn Mountains were occupied by glaciers during two widely separated glacial epochs." An area of about 300 square miles was covered with moving ice; and, at the time of maximum glaciation, it is probable that there was an almost continuous area of ice and snow over a region 40 miles long and 27 miles wide. This glaciation has been referred definitely to the last glacial epoch.

### History

1. PERIOD OF EARLY EXPLORATION.—Early accounts of the Black Hills deal chiefly with general descriptions of the country traversed. They emphasize the topographic, geologic, and humanistic features, but contain little of scientific value concerning the plant life of the region. In 1803 the first governmental expedition into the northwest was organized, under the leadership of Captains LEWIS and CLARK. This expedition did not visit the Black Hills, but referred to them in their report.

In 1810 the HUNT expedition left the Missouri River, and traveled overland on a journey to Astoria near the mouth of the Columbia River, probably traversing a part of the Black Hills. The discovery of mammalian remains in the Bad Lands of the White River country resulted in a series of expeditions into that region.

2. PERIOD OF MILITARY EXPLORATION.—In 1855 the Sioux expedition into the Dakota country was made under the command of General W. S. HARNEY, and in the accompanying geological report HAYDEN makes several references to the flora of the region. Further explorations by WARREN and HAYDEN were carried on in 1857, when they practically encircled the Hills. HAYDEN again visited the Hills in 1859, as the geologist in the expedition led by RAYNOLDS (21), to explore the region of the Yellowstone River and its tributaries. In the report of his geological findings (12), which he presented in 1861, there is a list of the flora of the upper Missouri region, prepared by GEORGE ENGELMANN, which is probably the first taxonomic work referring to the Black Hills. A list of 721 plants is reported, with approximate ranges in some instances, and the points of collection in others. Of these, 34 are specifically referred to the Hills, and 76 are noted as species from the Bad Lands.



In 1874 CUSTER (7) organized an expedition at "Fort Abraham Lincoln, Dakota Territory for the purpose of reconnoitering a route from that post to near Bear Butte in the Black Hills, and exploring the country south, southeast and southwest of that point." This expedition is of particular interest, as it was the first one to penetrate the interior of the region. DONALDSON, the botanist of the expedition, estimated the number of flowers in bloom in Floral Valley at 50, while an equal number of varieties had bloomed or were yet to bloom. The number of trees, shrubs, and grasses was estimated at 25, making a total flora of the valley embrace 125 species. LUDLOW (13) accompanied the CUSTER expedition as engineer officer. His report includes two lists of plants, which are the first that can definitely be related to the Hills proper. The first, a list of the trees and shrubs, was compiled by N. H. WINCHELL, and contains the names of 36 species. In it *Pinus resinosa* Ait. is erroneously listed as the only species of pine in the region. The second list includes the names of 75 plants, representing 23 families, which were collected by DONALDSON and determined by JOHN M. COULTER.

Reports of gold following CUSTER's expedition caused the government to send a geologic survey into the Hills the following year. This party was in the field for nearly five months, and made a careful survey of the entire area between the forks of the Cheyenne. JENNEY (18) was particularly impressed with the luxuriant growth of the grasses, the abundant timber, and the large number of wild fruits. He observed that the eastern aspect of the flora "bears quite a resemblance to that of southern Maine and New Hampshire in the same latitude." Although this expedition was primarily a geologic and topographic survey, a number of plants were collected and later determined by ASA GRAY. The NEWTON-JENNEY report (18) includes a list of 174 plants.

3. RECENT BOTANICAL STUDIES.—In 1892 RYDBERG was commissioned by the United States Department of Agriculture as an agent in the Division of Botany, for the purpose of making an investigation of the flora of the Black Hills. Prior to this time, such botanical work as had been done was purely incidental, and no serious attempt had been made to make an accurate survey of the

flora. The party spent nearly three months in the field and collected throughout the Hills.

In this report RYDBERG (22) divides the Black Hills into five floral districts: Foothills, Minnekahta Plains, Harney Mountain Range, Limestone District, and Northern Hills. Each district is discussed from the floristic standpoint, and the characteristic plants are noted with comments upon their ecology, geographic relationships, and taxonomic characters. The report contains a list of 688 species, representing 82 families, with data regarding the collection of each.

In 1895 WILLIAMS (31) published a list of trees and shrubs native to South Dakota, which included plants from the Black Hills. In 1898 BESSEY visited the Hills and investigated the occurrence of *Adiantum capillus-veneris* at Cascade. He concluded that it had not been introduced by human agency, but that it is indigenous along this warm stream. In 1899 SAUNDERS (24) published a catalogue of the ferns and flowering plants of South Dakota, which includes 508 species of Black Hills plants, and 144 others that might be referred to that region on the basis of the general range given, a total of 652 plants.

Since 1900 there have been several brief articles citing additions to the flora. VISHER (27-29) visited the Hills in 1908, 1911, and 1912, and made collections on each occasion. His published lists of additions to the flora include the names of 115 plants. In 1915 BALL (2) published an account of the willows of the Black Hills which summarized the results of personal collections made in 1908, 1910, and 1913. He lists 12 species of native willows, 3 of which range nearly across the continent. One is a distinctly eastern form, the Black Hills being its western limit; while 8 are western species, typical of the Rocky Mountains and westward, that have their eastern limits in the Hills.

In 1923, OVER (19) published a list of the trees and shrubs of South Dakota which gives the names and ranges of 85 species, including 14 introduced forms. Of these, 37 are referred to the Black Hills.

More recently (1926-1928) MCINTOSH (14-17) has investigated the flora of the Hills. The collection in the herbarium of the State

School of Mines at Rapid City, containing over 800 species of vascular plants, is largely the result of his personal work, and is the most extensive collection of plants of this region in existence. His first paper deals with the flora of Custer State Park. He lists the outstanding plants found in seven typical habitats: the foothills, the foothills streams, the mountains, the moist ravines, the valleys, the gorge, and the granite peaks. Among the 200 species of vascular plants listed are certain ones that are rare or peculiar to that part of the Hills, such as *Onoclea struthiopteris*, *Pinus flexilis*, *Viola blanda*, and *Myosotis alpestris*.

In a second paper he considers the sources of the flora of the Black Hills. On the basis of the distribution of 600 species, he finds that 6 per cent are common to the Northern Hemisphere, 16 per cent occur across North America, 7 per cent are of eastern origin, 17 per cent have come from the Great Plains, 36 per cent are of western origin, 6 per cent are from the north, 2 per cent have southern affinities, and 9 per cent are Old World weeds or escapes from cultivation.

A third paper deals with the Cascade Valley and vicinity, south of Hot Springs. In it he discusses the streamside vegetation, the inner valley, the main valley, the grassland, and sageland, noting the outstanding plants in each habitat.

## PART I. FLORISTICS

During the summers of 1926 and 1927, collections were made at approximately fifty stations in the Black Hills and adjacent areas (fig. 1). The stations were selected in such a way that both geographical and formational distributions of the habitats were secured, as it was felt that the studies would then be representative of the region, and that a true picture of the floristic and successional relationships could be developed. The habitats were divided into four groups, representing (1) the prairie grassland type, (2) the conifer or montane forest, (3) the foothills chaparral, and (4) the upland meadow. In the first group were seventeen widely distributed stations which completely encircled the Hills; the second included fourteen stations located throughout the central region; in the third were eight lower canyon and foothills habitats, dominated by oak and

other deciduous trees and shrubs; and the fourth contained five upland meadows located for the most part in the Limestone District. In addition to these, special studies were made of the vegetation of the three principal peaks, and of the streamside and lakeside flora.

The collection,<sup>3</sup> which includes 765 species representing 341 genera and 91 families, doubtless contains the characteristic elements of the flora of the Black Hills, and an analysis of it should present an accurate representation of the floristic situation. It is in no sense complete, however, and probably includes only about one-half of the fernworts and seed plants of the region. A careful cross-check of the published lists of GRAY (10), RYDBERG (22), SAUNDERS (24), VISHER (27-29), and MCINTOSH (14-17) with that of the writer gives a total of nearly 1200 plants. A complete catalogue of the region would undoubtedly exceed this figure, as there are some collections that have not been reported as yet,<sup>4</sup> and further additions to the known flora may be expected as the territory is more completely studied.

#### COMPOSITION OF FLORA

The dominant flora of the Black Hills proper includes relatively few tree forms, belonging to nine families. First in importance is the Pinaceae, represented by *Pinus scopulorum*, *P. murrayana*, *P. flexilis*, and *Picea albertiana*. The yellow pine is the outstanding tree of the hills, with the northern spruce occurring frequently at the higher elevations and in the northern valleys. The lodgepole pine is restricted to one point in the hills, near Nahant, although there are a few widely scattered single specimens reported for other locations; and the limber pine occurs only in the Needles region near Harney Peak, on the Custer-Pennington county line. It is to these conifers that the Black Hills owe their name, for the approaching traveler sees them at a distance rising from the prairie as a blue-black mass.

The Fagaceae, represented by *Quercus macrocarpa*, dominate the

<sup>3</sup> A complete collection of the specimens obtained in the Black Hills has been deposited in the herbarium of the Public Museum, Milwaukee, Wisconsin, and will be preserved as a Black Hills unit.

<sup>4</sup> OVER is working on a revised flora of South Dakota which he expects to publish at an early date. Since this paper was written, MCINTOSH (17) has published a list of additions to the flora containing the names of 240 species.

lower valleys on the eastern slopes; with it are commonly found members of the Ulmaceae, *Ulmus americana* and *Celtis occidentalis*, the latter being more frequent in the southern valleys. Other tree families represented in the region are the Oleaceae by *Fraxinus pennsylvanica*; the Aceraceae by *Acer negundo*; Pomaceae by *Ame-lanchier alnifolia*, *Crataegus sheridana*, and *Sorbus scopulina*; and the Drupaceae by *Prunus melanocarpa*, *P. virginiana*, *P. americana*, *P. pennsylvanica*, and the shrubby *P. besseyi*. The Salicaceae are well represented along the streams by several species of willows and poplars, and the Betulaceae by *Betula fontinalis* and *B. papyrifera*, the latter occurring commonly in the secondary succession.

Shrubs occupy an important place in the open woodlands, and are the dominant forms in the chaparral of the foothills. Rosaceous forms are numerous, the following species being especially prominent: *Cercocarpus parvifolius*, *Rosa arkansana*, *R. woodsii*, *Dasio-phora fruticosa*, *Physocarpus intermedius*, *Spiraea lucida*, *Rubus strigosus*, and *R. americanus*. *Rhus trilobata*, *R. rydbergii*, *Symphoricarpos occidentalis*, *S. racemosus*, *Shepherdia canadensis*, and *S. argentea* are very common in the drier situations; while *Sambucus racemosa*, *Viburnum pauciflorum*, *Cornus stolonifera*, and several species of *Ribes* are found in the more mesophytic habitats. *Ceanothus velutinus* occurs at the higher elevations, especially in the Limestone District.

Numerically, eight of the ninety-one families found in the region include over half of the total number of species, distributed as follows: Compositae 124, Gramineae 85, Leguminosae 56, Cyperaceae 36, Rosaceae 31, Ranunculaceae 26, Cruciferae 25, and Scrophulariaceae 20. The Compositae and Leguminosae are associated chiefly with the grassland formation, and show decided plains and western affinities. The grasses dominate the foothills and meadows, and penetrate into the yellow pine savannah and the oak chaparral. The sedges are found mainly with the mesophytic grasses in the upland meadows, although there are a few of the more xerophytic species that occur regularly in the prairie habitats. The Rosaceae are nearly 50 per cent western, and constitute an important element in the chaparral and open forest. The Ranunculaceae and Scrophulariaceae show no outstanding affinities, and the Cruciferae owe their numeri-

cal rank to the presence of a large number of ruderal forms in the Hills.

Other families that deserve special mention are the Orchidaceae and Euphorbiaceae. Eleven species representative of the former are distinctly northern, and are found in the cooler situations in the northern hills. The representatives of the latter family are southern, more than half of them being typical species of the south and southwest.

#### GEOGRAPHIC RELATIONSHIPS

The isolated position of the Black Hills suggests the question of the sources of the flora. The ranges of 750 plants were determined, and they were then grouped into the following classes: (1) cosmopolitan species, (2) species ranging across the Northern Hemisphere, (3) species ranging across North America, (4) species proper to the Great Plains, (5) western species, (6) eastern species, (7) northern species, (8) southern species, (9) endemic species, and (10) ruderal species and Old World forms escaped from cultivation. This classification was used by MCINTOSH (15) in his study, and has been followed in order to make the results comparative.

Slightly in excess of 26 per cent of the species are characteristic of the Great Plains. This is what might be expected, as the plains completely surround the Hills, making contacts with the foothill chaparral and savannah associations; and, in many instances, penetrate the central regions and form prairie areas in the heart of the forest.

Species that are characteristically western comprise 25 per cent of the total. These may be divided into two groups: (1) those found with the western yellow pine and typical of the Rocky Mountains, and (2) those species that have migrated into the Hills from the western plains and foothills. In the first group are *Berberis aquifolium*, *Castilleja sulphurea*, *Frasera speciosa*, *Thalictrum venulosum*, *Lilium montanum*, *Geranium richardsonii*, *Vaccinium scoparium*, *Clematis pseudoalpina tenuiloba*, *Arnica cordifolia*, and *Ribes inerme*; the second category includes *Aristida fendleriana*, *Koeleria cristata*, *Sitanion hystrix*, *Carex filifolia*, *Artemisia tridentata*, *Lappula floribunda*, *Gaillardia aristata*, *Calochortus nuttallii*, *Rhus trilobata*, and *Cercocarpus parvifolius*.

Approximately 9 per cent of the species are typically eastern. They are usually found associated with *Quercus macrocarpa*, and include the following representative forms: *Ulmus americana*, *Acer negundo*, *Prunus virginiana*, *Celtis occidentalis*, *Aquilegia canadensis*, *Viola pubescens*, *Sanguinaria canadensis*, *Actaea rubra*, *Fragaria americana*, and *Unifolium* (*Maianthemum*) *canadense*.

Species that are characteristic of the north constitute about 6 per cent of the flora. In the yellow pine and spruce associations of the northern hills are found *Betula papyrifera*, *Viburnum pauciflorum*, *Linnaea americana*, *Gentiana plebeja*, *Circaea alpina*, *Calypso bulbosa*, *Limnorchis borealis*, *Moneses uniflora*, and *Corallorrhiza trifida*. In the upland meadows of the Limestone District are *Carex tenera*, *C. aenea*, *Torresia odorata*, *Calamagrostis canadensis*, *Juncus ensifolius*, and *Astragalus alpinus*.

Southern species that occur in the Hills comprise 5 per cent of the total. They are associated with the grassland and scrub formations, and for the most part are herbaceous forms that have migrated northward across the plains from the southwest. In this group are *Artemisia filifolia*, *Ipomoea leptophylla*, *Croton texensis*, *Euphorbia arkansana*, *E. dentata*, *Salvia lanceolata*, *Allionia linearis*, *Gaura parviflora*, *G. coccinea*, *Argemone intermedia*, *Aristida longiseta*, *Polygala alba*, and *Verbena bipinnatifida*.

As in most regions, a large number of the plants represent species of wide distribution. This group includes about 22 per cent of the flora, and may be subdivided further into three classes: (1) species that range across North America, comprising 17 per cent of the total flora; (2) species that extend across the Northern Hemisphere, 4.5 per cent; and (3) a few species that are truly cosmopolitan in character, 0.5 per cent. In the first class are such representative forms as *Populus tremuloides*, *Sambucus racemosa*, *Symphoricarpos racemosus*, *Cornus canadensis*, *Chamaenerion angustifolium*, *Anemone cylindrica*, *Salix fluviatilis*, *Viola adunca*, several species of *Carex*, *Pyrola*, *Equisetum*, and many grasses. In the second class are *Sagina saginoides*, *Eleocharis glaucescens*, *Triglochin palustris*, *Potamogeton pectinatus*, *Juniperus communis*, *Galium boreale*, *Limosella aquatica*, and several fernworts. Cosmopolitans occurring in the region in-

clude *Botrychium virginianum*, *Filix fragilis*, *Callitriche palustris*, and *Typha angustifolia*.

Ruderals are becoming increasingly common in the Black Hills, and comprise 6 per cent of the flora. These owe their presence in the territory primarily to the activity of man. Many are escapes from cultivation, others are ballast plants that have come in with the advent of the railroad, and a large number have been introduced with agricultural seeds. Without doubt several species have been able to invade the region because of superior devices for migration, a short vegetative cycle, and a structural organization that made ecesis possible. In this connection, it is of interest to note that 5 per cent of the additions to the flora reported in this paper are ruderal plants. This does not necessarily mean that these species have come into the region since the publication of previous lists. Some have doubtless done so, while others have become sufficiently widespread in their distribution to come into the hands of the collector.

The geographic grouping of the flora already indicated agrees essentially with the analysis made by McINTOSH, except for the larger percentage of western species and a somewhat smaller proportion of southern ones reported in his paper. The fact that the present study included a number of outlying foothills habitats, dominated by species characteristic of the plains, would account for the larger percentage in that group and the relatively lower percentage of western forms.

The impression given by the preceding account of the floristic composition of the Black Hills would be extremely misleading without further analysis, based upon the local distribution of the plants in the various habitats studied. Since these will be discussed more completely later, four habitats representing wide divergences in floristic make-up will illustrate this detail of the situation.

The first habitat is known as Dark Canyon, located about 5 miles up Rapid Canyon from the Municipal Camp at Rapid City. It is a typical rocky canyon having precipitous sides abounding in crevice plants and topped by yellow pine. The floor of the canyon is occupied by oak, elm, boxelder, hackberry, several species of plums, and



various shrubs and herbaceous forms. Of 60 plants collected in this habitat, 30 per cent were eastern, 22 per cent North American, 18 per cent western, 12 per cent Great Plains, and 10 per cent northern.



FIG. 7.—Spearfish Canyon, showing northern spruce association

The remaining 8 per cent consisted of a few widespread forms and one species of *Aquilegia* that appears to be a new form.

Spearfish Canyon, from Savoy to a point approximately 4 miles above Cheyenne Crossing, is the second habitat. It is dominated by the yellow pine, with the northern spruce becoming more abundant upstream, and even assuming the dominant rôle on the north-facing

slopes (fig. 7). The habitat is cool and mesophytic, and the underflora is rich in northern and eastern species. A total of 126 plants was collected along this stream, with the following geographic distribution: northern forms 27 per cent, eastern 22 per cent, North American 22 per cent, western 20 per cent, Northern Hemisphere 5 per cent, Great Plains 1.5 per cent, and cosmopolitan 1.5 per cent.

Reynolds Prairie is representative of the grassland formation. Lying in the west-central portion of the Hills, and surrounded by towering limestone cliffs on the west and the yellow pine forest on



FIG. 8.—Reynolds Prairie: typical grassland association in the west-central hills; rim of Limestone Plateau seen in background.

the east, it presents a flora that is typical of the prairies and plains of the west (fig. 8). Of 50 species collected, 42 per cent were Great Plains types, 36 per cent western, 8 per cent North American; 6 per cent eastern, 4 per cent northern, and 4 per cent Northern Hemisphere.

The last illustration is drawn from a study made of the chaparral of the western foothills at a point near Newcastle, Wyoming (fig. 9). *Cercocarpus parvifolius* dominates, with other shrubs and herbs playing a minor rôle in the association. A total of 60 species was collected, of which 56 per cent were forms characteristic of the Great Plains, 25 per cent western, 10 per cent southern, 5 per cent North American, and 3 per cent northern; eastern species were entirely lacking.

Table I summarizes the results of the four studies. The contrasts are as striking as the figures indicate. In one habitat it would be easy to imagine one's self transplanted to a woodland dell in an eastern deciduous forest, another suggests the montane forest of the Rocky Mountains, and a third the open prairie. Thus, although there is no true floristic unity in the Hills, and although it is actually a meeting place of plants from all points of the compass, there still remains a



FIG. 9.—Chaparral formation near Newcastle, Wyoming, with *Cercocarpus parvifolius* dominating; zonation between chaparral and grassland very clearly defined at this point.

semblance of formational unity when the analysis is carried out on that basis. It is not a hopeless jumble, as one might conclude from the general analysis of the flora, but rather a vivid illustration of the fact that plants persist in habitats that are within the physiological limits of their range of adjustment, regardless of geographic affinities. The mesophytes from north, east, and west meet in the pine-spruce forest, and the xerophytes of the south mingle with those of the west and east on the foothills and prairies.

#### DISJUNCT SPECIES

In line with the general complexity of the flora is the occurrence of a large number of disjunct species. It would not be inaccurate to

consider all the tree forms as such, inasmuch as the Hills are completely encircled by grasslands; and it might further be argued with some justification that, by the same fact, all the plants of the Black Hills are disjuncts except those proper to the Great Plains. If the region is regarded as a great floristic island, isolated by climatic barriers and harboring elements from all points, then this conception is the correct one, and only those forms should be classed as continuous species that are now found in the intervening prairies and plains.

Thus the dominant yellow pine is disjunct from its main range in the Rocky Mountains; and other trees of more limited distribution, such as the bur oak, white spruce, lodgepole pine, limber pine,

TABLE I

HABITAT	DOMINANT VEGETATION	No. OF SP.	GEOGRAPHIC AFFINITIES, PERCENTAGES							
			G.P.	W.	E.	N.	S	N.A.	N.H.	Cos.
Dark Canyon.	Oak	60	12.0	18.0	30.0	10.0	0	22.0	3.0	3.0
Spearfish Creek. . . . .	Pine-spruce	126	1.5	20.0	22.0	27.0	0	22.0	5.0	1.5
Reynolds Prairie. . . . .	Grasses	50	42.0	36.0	6.0	4.0	0	8.0	4.0	0
Newcastle. . . .	Cercocarpus	60	56.0	25.0	0	3.0	10.0	5.0	0	0

and others previously noted fall in the same category. The last two pines may be considered as local disjuncts as well as regional ones, since there are no special barriers to prevent extension of their ranges within the limits of the Hills; and the same term can be applied to many other species occurring in the region.

Among the herbaceous plants, one of the striking disjuncts is *Calypso bulbosa*. Two specimens of this dainty flower were found in the cool spruce forest along Spearfish Creek above Cheyenne Crossing. This is apparently the first time that this orchid has been found in the Hills. *Limnorchis borealis* grows in the same habitat, and *L. media* occurs in lower Spearfish Canyon. *Piperia unalaschensis* was found at two widely separated points, near Crooks Tower on the Limestone Plateau and on the hills above Pinecrest Park near Deadwood. *Viola bellidifolia* occurs in Dark Canyon, and *V. renifolia* was found only in upper Spearfish Canyon. The former is a western form, while the latter is distinctly northeastern.

Local disjunction is most strikingly illustrated in the case of the fernworts. Thus *Botrychium virginianum* is known to occur only in the vicinity of Deadwood near Pinecrest Park; *B. lanceolatum* in Spearfish Canyon above Savoy; and *B. neglectum*, according to RYDBERG, occurs south of Custer in the central hills. *Onoclea sensibilis* and *O. struthiopteris* are found on Squaw Creek just below the State Game Lodge, while *Adiantum modestum*<sup>5</sup> is indigenous along the banks of Cascade Creek south of Hot Springs.

The question which the occurrence of such a number of disjuncts raises is one of interpretation. What is the ecological significance of disjunction, and on what basis can it be explained? This problem can best be considered by grouping disjuncts into three classes: (1) habitat, (2) relict, and (3) pioneer or invader disjuncts.

In the first group are those forms that are disjunct solely because the habitat in which they can exist is itself discontinuous. In other words, the basis for disjunction is physiographic. This is well illustrated in the case of many of the more common hydrophytes that are found widely distributed in nature but are locally restricted to water holes, ponds, and lakes. Examples of this type of disjunct in the Black Hills are such forms as *Typha angustifolia*, occurring at Sylvan Lake; *Polygonum amphibium*, found in a water hole near Wind Cave; and *Marsilea vestita*, which occurs in water holes along the eastern foothills and in similar situations in the eastern part of the state.

Habitat disjunction may be edaphic rather than physiographic. This would occur where there are locally distributed areas that are strongly alkaline or acid in character. In the former the saline character of the habitat is the limiting factor, and acts to exclude all plants that lack salt tolerance. Examples of this type are not numerous, but the occurrence of an almost pure stand of *Distichlis spicata* in Hell Canyon is a good local illustration.

The relict disjuncts present quite a different situation. The group is interpreted as being a last outpost of a once more widespread distribution, which has survived in the face of changing conditions and the consequent shift in the character of the plant population.

<sup>5</sup> This fern was first reported by BESSEY as *A. capillus-veneris* L. BOT. GAZ. 26: 211. 1898.

Discontinuity may be the result of changes in the intervening portions of the original range which did not affect the local habitat. In this case the relict may be able to resist competition successfully within the new limits established; but it will be unable to bridge the gap to its main range and reestablish continuity.

It is not easy to establish with certainty the past history of this type of disjunct, since the explanation involves a knowledge of prehistoric climatic changes that can only be determined on the basis of indirect evidence. In the case of the glacial relict there is the best opportunity for interpretation, since the limits of glaciation are fairly well defined, and the effect on the climate and vegetation can be rather accurately estimated. The northern white spruce is the best illustration of this type in the Black Hills, although many of the northern herbs, especially the bog orchids, doubtless owe their present isolation to the effect of glacial activity and subsequent climatic changes.

The pioneer or invader disjuncts represent the other extreme in plant dynamics. In general plant invasion is continuous and recession discontinuous, but this is not necessarily so. When the devices and agencies of plant migration are considered, it is not surprising to find new invaders that have successfully established themselves, and are at the same time entirely discontinuous from their main range. Prominent in this group are the ferns, and many of the ruderal and Old World forms that are becoming increasingly common in the Hills.

#### VEGETATIONAL HISTORY

It is not within the scope of this paper to inquire into the past vegetational history of the Black Hills, prior to the advent of the present flora,<sup>6</sup> but the presence of such a diversity of floristic elements in the Hills today raises the question as to their probable origins. Why are the eastern bur oak, the northern spruce, the western pines, and the various southern species in the region, and what was the sequence of their arrival?

<sup>6</sup> For a discussion of the fossil plants of the Black Hills, see WARD, L. F., Cretaceous formations in the Black Hills as indicated by fossil plants. U.S. Geog. Survey, 19th Ann. Rept. pt. II. pp. 521-946. 1899; also WIELAND, G. R., American fossil cycads. vol. II. Taxonomy. Carnegie Inst. Washington Publ. 34. p. 95. 1916.

COWLES (6), CLEMENTS (4), ADAMS (1), TRANSEAU (25), GLEASON (9), and many others have considered the problems of plant migration and succession, and as a result the essential principles of plant dynamics are generally understood. Much remains to be done in the field of prehistoric vegetational studies, however, and the complete story must await the fuller details which the paleobotanist and geologist may be expected to contribute, as research in these fields is continued. Until we have a more complete picture of past floras, especially of the more recent geologic periods, much of the story must be conjectural; but the body of evidence is sufficiently great to justify attempts to reconstruct the floristic record.

The significant feature of preglacial time, as it relates to present day floras, was the uplift of the Cordilleran Mountains which took place during Tertiary time. This had a profound effect upon the vegetational history of North America, as HARVEY (11) has pointed out. If the Cretaceous vegetation were somewhat uniform in its distribution, as is generally believed, the elevation of the Rocky Mountains, with the consequent aridity to the east, must be considered as the basic cause of vegetational segregation, which has resulted in the formation of the Great Plains and the north-south cleavage of the great forest centers of North America. According to GLEASON, the second cleavage was probably coincident with the approach of the first glacial period, and continued during the ice age. This led to the separation of the northern coniferous forests from the southern flora dominated by angiosperms.

Thus, during the glacial epoch, the climate of the Black Hills was undoubtedly favorable for the northern conifers, because of the southward extension of the invading ice sheet, and it was at that time that the northern spruce extended its range into the region. With the advent of milder and drier conditions, during the xerothermic period of Post-Wisconsin times, marked shifts in the distribution of the floral groups occurred. The coniferous belt moved northward and westward as the ice sheet receded, giving way to the prairie flora in the Great Plains and to the deciduous forests in the eastern United States. This movement resulted in the isolation of relict conifers in the more favorable habitats, such as the Black Hills. It seems

probable that during glacial time the spruce was the dominant tree in the Hills.

The invasion of the more xerophytic yellow pine, lodgepole pine, and limber pine from the west followed as the climate became drier and warmer. Finally the spruce continued to persist only at the higher elevations and in the colder valleys where conditions were favorable, and the yellow pine occupied the major portion of the central hills region.

Continued and probably increasing aridity favored further advances of the flora northward and eastward, resulting in the isolation of the Black Hills from the montane forest of the Rocky Mountains, and the invasion of the eastern deciduous forests. Some of the southern species may have established themselves in the Hills at this time, while others are the result of a more recent migration.

If postglacial climate had been uniform up to the present time, it would be difficult to account for the eastern elements in the region; but the investigations of BLYTT, ANDERSSON, SERNANDER, GEIKIE, and others, while not in agreement as to the details, indicate that there have been alternating periods of great climatic diversity since the last glacial epoch.<sup>7</sup> It is probable that there was a gradual change from the xerothermic conditions of early postglacial time to a more humid climate. This halted the invasion of the prairie into the forest, and swung the balance in the other direction. As a result there was an extension of the deciduous forest to the west and northwest along the river bottoms and adjacent highlands. The oak, elm, ash, boxelder, hackberry, and cottonwoods probably migrated up the Missouri and its tributary streams, and thus reached the Black Hills during the humid period.

The present isolation of these trees remains to be explained. The species found in the Hills also occur in the eastern part of the state, and many of them are distributed locally along the intervening streams; but there is little suggestion of continuity at the present time. Two explanations may be offered. The first is that the climatic cycle has swung around to another drier period, bringing with it

<sup>7</sup> For a summary of the work on postglacial climate, see CLEMENTS, F. E., *Plant succession*. Carnegie Inst. Washington Publ. 242. pp. 377-403. 1916.



conditions unfavorable for tree growth. This has favored an extension of the prairie grassland, and has restricted the tree forms to the more mesophytic locations in the river bottoms. The second possibility is that of human influence. If the tension line existing between the grassland and the forest were very finely drawn, any disturbance of this balance would react to the advantage of one over the other. Thus repeated burning of the prairie by the Indian, and deforestation by the pioneer, would tend to favor the gradual diminution of the deciduous forest. When the climate of the region west of the Missouri is taken into consideration, the first explanation appears to be the more satisfactory one; but humanistic factors were doubtless effective in hastening the process of isolation.

#### ENDEMISM

The question of endemism is always a difficult one. In a region that is definitely isolated, such as an oceanic island, the issue is obvious; but in the case of one that is a part of a continental area, it is always possible that the endemic plant may be in reality a disjunct of such infrequent occurrence that other stations have not been reported. Furthermore, the endemic may be only a variety of a more widespread species which appears temporarily in a habitat, due to unusual environmental conditions. It is desirable, therefore, that statements regarding new and endemic species should be extremely conservative and provisional. It seems likely that there are a few endemic species in the Black Hills, but definite confirmation must await the test of time. The following new species, which may possibly be endemic, have been determined tentatively by AVEN NELSON: *Aquilegia tenebrosa* A. Nels., *Lesquerella alpina argillosa* A. Nels., and *Solidago sigmoidea* A. Nels.

#### ADDITIONS TO FLORA AND EXTENSIONS OF KNOWN RANGES

The collections made in connection with these studies include a number of species that have not been reported in the previous lists of GRAY, RYDBERG, SAUNDERS, VISHER, BALL, and MCINTOSH. Where the addition also extends the known range of the species, the name is starred and the previous range indicated.<sup>8</sup>

<sup>8</sup> The ranges given are taken from RYDBERG, P. A., *Flora of the Rocky Mountains and adjacent plains*. New York. 1922.

- Botrychium lanceolatum* (Gmel.) Angstr., Spearfish Creek  
*Equisetum kansanum* Schaffn., Hot Springs  
*Equisetum scirpoides* Michx., Spearfish Canyon  
*Typha angustifolia* L., Sylvan Lake  
*Lophotocarpus depauperatus* (Engelm.) J. G. Sm., Water hole near Fairburn  
*Aristida longiseta robusta* Merr., Hot Springs  
*Stipa columbiana* Macoun., \* Deadwood (B.C.-Wyo.)  
*Calamagrostis purpurascens* R.Br., Harney Peak  
*Aira caespitosa* L., Inyankara Creek  
*Danthonia intermedia* Vasey, Limestone District  
*Poa longipedunculata* Scribn., \* Whitewood (Wyo.-N.M.-Utah)  
    " *lucida* Vasey, Common  
    " *palustris* L., Spearfish, Whitewood, Boxelder Creek  
    " *trivialis* L., Custer Peak and Deadwood  
*Bromus japonicus* Thunb., \* Belle Fourche, Beulah, Hot Springs (Adv. from Eu.)  
*Agropyron dasystachyum* (Hook.) Scribn., Reynolds Prairie  
*Agropyron riparium* S. & S., \* Newcastle (Mont.-Colo.)  
*Hordeum pusillum* Nutt., Buffalo Gap, Edgemont  
*Scirpus atrocinctus* Fernald, Sylvan Lake  
*Carex abbreviata* Prescott, Head of French Creek, Limestone District  
    " *aenea* Fernald, Limestone District  
    " *bebbii* Olney, Boulder Canyon, Boxelder Creek  
    " *concinna* R.Br., \* Spearfish Canyon (Rare in this range)  
    " *eburnea* Boott., Boulder Canyon, upper Spearfish Canyon  
    " *hoodii* Boott., Spearfish Canyon, Terry Peak, Limestone District  
    " *obtusata* Lilj., Boulder Park, Terry Peak  
    " *rosea* Schk., Rapid Creek and Dark Canyon  
    " *rupestris* All., \* Harney region (Rare alpine form)  
    " *scoparia* Schk., Sylvan Lake, French Creek  
    " *xerantica* Bailey, Limestone District  
*Tradescantia bracteata* Small., Rapid City, Hermosa  
*Tradescantia laramiensis* L. N. Goodding, \* Hot Springs (Mont.-Col.-Utah)

- Zygadenus intermedius* Rydb.,\* Common (Colo.-Wyo.)  
*Calypso bulbosa* (L.) Oakes., Upper Spearfish Canyon (Very rare)  
*Limnorchis borealis* (Cham.) Rydb., Spearfish Canyon (Rare)  
*Piperia unalaschensis* (Spreng.) Rydb.,\* Deadwood, Limestone District (Mont.-Colo.-Calif.-B.C.)  
*Corallorrhiza mertensiana* Bong.,\* Spearfish Canyon, Limestone District (Alaska-Mont.-Ida.-Calif.)  
*Populus candicans* Michx., Rapid City  
*Ulmus fulva* Michx., Hot Springs (Doubtful, identified without fruit)  
*Celtis crassifolia* Lam., Hot Springs  
*Urtica viridis* Rydb., Spearfish Canyon  
*Polygonum pratincola* Greene., Needles Highway  
*Chenopodium paganum* Reichenb., Ruderal (Adv. from Eu.)  
*Chenopodium subglabrum* (Wats.) A. Nels., Edgemont  
*Amaranthus powellii* S. Wats., Needles Highway  
*Montia parviflora depressa* Robins, Whitewood Canyon  
*Lychnis alba* Mill., Ruderal (Adv. from Eu.)  
*Stellaria crassifolia* Ehrh., Upper Spearfish Canyon  
*Cerastium elongatum* Pursh.,\* Harney Peak (Mont.-No. Wyo.-Wash.)  
*Aquilegia tenebrosa* A. Nels., n. sp., Dark Canyon  
*Batrachium flaccidum* (Pers.) Rupr., Keystone  
*Thalictrum nelsonii* Greene., Hot Springs, Inyankara and Boxelder Creeks  
*Arabis fendleri* (Gray) Greene, Custer Peak  
*Erysimum tener* A. Nels., Hot Springs (New species in ed.)  
*Lesquerella alpina argillosa* A. Nels., Hermosa (New variety)  
*Draba nitida* Greene, Boulder Park  
*Heuchera richardsonii* R.Br., Common  
*Ribes hudsonianum* Rich., Dark Canyon  
*Ribes irriguum* Dougl.,\* Dark Canyon, Boxelder Creek, Spearfish Canyon (B.C.-Mont.-Ida.-Ore.)  
*Potentilla ambigens* Greene.,\* Common (N.M.-Wyo.)  
*Potentilla paucijuga* Rydb.,\* Boulder Park (S.E. Utah)  
*Potentilla viridescens* Rydb., Inyankara Creek  
*Amelanchier oreophila* A. Nels., Limestone District

- Astragalus elatiocarpus* Sheld., Buffalo Gap  
*Oxytropis angustata* (Rydb.) A. Nels., Hermosa, Fairburn, Buffalo Gap.  
*Amorpha fragnans* Sweet., Hot Springs  
*Petalostemon mollis* Rydb.,\* Belle Fourche (Mont.-Colo.)  
*Meibomia canadensis* (L.) Kuntze., Boulder Canyon  
*Vicia cracca* L., Inyankara (Nat. from Europe)  
*Linum aristatum* Engelm.,\* Hot Springs (Greatly extends range)  
*Euphorbia arkansana* Eng. & Gray., Belle Fourche, Wind Cave  
*Rhus osterhoutii* Rydb.,\* Hot Springs (Colo.)  
*Rhus oxyacanthoides* (Greene) Rydb., Spearfish Creek  
*Rhamnus alnifolia* L'Her., Bear Butte Creek  
*Ceanothus sanguineus* Pursh.,\* Spearfish Canyon (Mont.-No. Cal.-B.C.)  
*Viola renifolia* Gray, Upper Spearfish Canyon  
*Epilobium saximontanum* Haussk.,\* Squaw Creek (Alta.-Wyo.-B.C.)  
*Oenothera muricata* L., Spearfish Canyon, Harney Region, Hill City  
*Gaura glabra* Lehm., Common on dry foothills  
*Pyrola minor* L., Nahant and Harney region  
*Moneses uniflora* (L.) Gray, Spearfish Canyon, Castle Creek  
*Androsace diffusa* Small, Boulder Park, Wind Cave, Custer Peak  
*Convolvulus ambigens* House, Beulah  
*Cryptantha crassiseppala* (T. & G.) Greene, Wind Cave  
*Mentha mollis* L., Spearfish Canyon, Deadwood  
*Monarda ramaleyi* A. Nels.,\* Whitewood (Colo.)  
*Physalis subglabrata* Mack., Hot Springs  
*Limosella aquatica* L., Limestone District  
*Galium vaillantii* DC., Hot Springs  
*Sambucus melanocarpa* Gray, Boulder Canyon  
*Gutierrezia microcephala* Gray,\* Hot Springs (Colo.-Tex. and westward)  
*Grindelia erecta* A. Nels., Newcastle  
*Chrysopsis bakeri* Greene,\* Sylvan Lake, Hot Springs (Wyo.-Ida.-N.M.-Utah)  
*Solidago sigmoidea* A. Nels., n. sp., Limestone District  
*Solidago trinervata* Greene, Belle Fourche, Spearfish, Sylvan Lake

*Aster oblongifolius rigidulus* Gray, Belle Fourche, Beulah, Hot Springs

*Erigeron concinnus* (H. & A.) T. & G., Beulah

“ *engelmannii* A. Nels., Hot Springs

“ *laetevirens* Rydb., \* Beulah, Buffalo Gap, Newcastle (Wyo.)

“ *montanensis* Rydb., \* Common (Mont.-Wyo.)

“ *speciosus* DC., Boxelder Creek

*Antennaria howellii* Greene, \* Common (Alta.-Mont.-Ida.-Wash.-B.C.)

*Antennaria marginata* Greene, \* Harney region (N.M.-So. Colo.-Utah.-Ariz.)

*Antennaria oxyphylla* Greene, Sylvan Lake

*Helianthus giganteus* L., Dry Gulch Rgr. Sta., Hot Springs

*Helianthus subtuberosus* Bourgeau, Needles Highway

*Bidens prionophylla* Greene, Hill City

*Artemisia gnaphalodes diversifolia* A. Nels., Belle Fourche

*Artemisia purshiana* Bessey, Deadwood, Hot Springs

*Arnica rydbergii dubia* A. Nels., Dark Canyon, Deadwood

*Senecio fendleri* Gray, \* Rapid City, Hermosa, Boulder Park (N.M.-Colo.-Utah)

*Senecio fendleri mutabile* Greene, Beulah, Harney region, Limestone District

*Tragopogon porrifolius* L., Escape from cultivation

*Hieracium scabriusculum* Schwein., Boxelder Creek

*Lactuca virosa* L., Belle Fourche, Hot Springs (Nat. or adv. from Eu.)

*Agoseris purpurea* (Gray) Greene, Terry Peak, Rare (Colo.-N.M.-Ariz.-Utah)

## PART II. SUCCESSIONAL STUDIES

The successional relationships in the Black Hills are intricate, as might be expected in a region having the floristic complexity indicated in the preceding section of this paper. It is desirable, therefore, to correlate the isolated units of vegetation in the region with the major formations of the western United States. Three major climaxes are generally recognized: (1) the grassland, (2) the scrub, and (3) the forest climax. Each is dominated by the life form

indicated, and is further subdivided into primary units, the formations, which in turn include one or more associations.

The formation is a climatic and physiographic unit which is characterized by one life form, namely, herb, shrub, or tree; but there need not necessarily be floristic unity. The association differs from the formation in this respect, and is here understood to be a subdivision of the latter having floristic unity. It is characterized by one or more dominant species, with which are associated numerous subdominants. These constitute the societies, and may be locally abundant, completely dominating limited areas within the association. The society is not an integral part of the association, however, but may occur in several associations within a formation. Further, the society commonly varies from the life form of the dominant species of the association. Thus there may be shrub societies in a grassland association, and herbaceous societies in the montane forest.

If the dynamic progress of vegetational development consists of a series of stages culminating in the formation, it is probable that a given region will contain units of vegetation that represent beginning and intermediate stages in such a succession. It is necessary, therefore, to include in a successional analysis a statement regarding the preclimax stages found, and to relate them to the climaxes toward which they are apparently developing.

In addition to the climax and preclimax stages, two others occur in the Black Hills. The first is the subclimax stage. This is preclimax in the succession; but it is sufficiently permanent to warrant special classification, since in many cases it has many of the characteristics of a climax situation. The second is the postclimax stage. This is represented by certain relict dominants, and is particularly significant because it indicates something of the past climaxes of the region.

#### METHOD OF TREATMENT

In arranging a scheme of classification on the basis of the preceding, it has seemed advisable to group together the climax, postclimax, and subclimax stages under the three major formations, even though the last two are not strictly climax units; and to consider

separately the preclimax or developmental stages. This treatment permits a discussion of the principal vegetational units in the order of their occurrence, from the prairie to the central hills; and, in consequence, should give a more understandable picture of the sequence of associations and the contacts existing between them.

### Synopsis of successional relationships

- I. Climax and late successional stages
  1. Grassland climax
    - a. Mixed prairie association
  2. Scrub climaxes
    - a. Sagebrush subclimax; *Artemisia* association
    - b. Petran chaparral climax; *Cercocarpus*-*Rhus* association
    - c. Chaparral subclimax; *Quercus*-*Rhus* association
  3. Forest climaxes
    - a. Deciduous forest postclimax; *Quercus* association
    - b. Petran montane forest climax; *Pinus scopulorum* association
    - c. Boreal forest postclimax; *Picea albertiana* association
    - d. Secondary succession subclimax
      1. *Pinus murrayana* association
      2. *Betula*-*Populus* association
- II. Preclimax or developmental stages
  1. Hydric series
    - a. Pond stage
    - b. Streamside stage; *Betula*-*Salix* association
    - c. Upland meadow stage; Grass-sedge association
  2. Xeric series
    - a. Rock pioneer stage
    - b. Erosion pioneer stage
    - c. Secondary succession pioneer stage

### I. Climax and late successional stages

#### GRASSLAND CLIMAX

MIXED PRAIRIE ASSOCIATION.—The grassland climax is represented in the Black Hills and adjacent plains by the mixed prairie association. Studies of this type were made at Sturgis, Rapid City,

Hermosa, Fairburn, Buffalo Gap, Hot Springs, Edgemont, Hell Canyon, Inyankara, Beulah, Belle Fourche, Wind Cave, Reynolds Prairie, and Boulder Park. The last two are in the Hills proper; the others are habitats where the prairie extends up to and makes contact with the chaparral, deciduous forest, or yellow pine on the foothills (fig. 10). Available data for nine of these stations give an



FIG. 10.—Contact zone between yellow pine and mixed prairie in northern foothills; the yellow pine extends down rocky ridges.

average annual rainfall of 17.56 inches, the precipitation being greater on the eastern foothills than on the western, where it falls below 16 inches.

As the name suggests, the mixed prairie represents a grassland association in which the tall grasses of the true prairie and the short bunch grasses of the plains intermix and maintain equilibrium, the latter occurring as an understory of the former. Thus, in the habitats studied, the prairie dominants *Koeleria cristata*, *Stipa comata*, *Agropyron smilhii*, and *A. spicatum* occur as codominants with the short grasses and sedges *Bulbils dactyloides*, *Bouteloua gracilis*, *Carex stenophylla*, and *C. filifolia*. *Koeleria cristata* is the most abundant of the prairie grasses, occurring in thirteen of the fourteen habitats



studied. *Stipa comata* is one of the common dominants, and *S. viridula* is occasionally found with it. *Agropyron* is much less common than either of the two preceding genera. *Bulbilis dactyloides* is the most abundant of the short grasses, with *Bouteloua gracilis* second in importance. At least one of the two species of *Carex* occurs at ten of the stations. The most common groupings of the dominants are: *Koeleria-Stipa-Bulbilis*; *Koeleria-Stipa-Agropyron-Bouteloua-Carex*; *Stipa-Bouteloua-Carex*; *Koeleria-Bouteloua-Bulbilis*; and *Koeleria-Bouteloua*.

In addition to the dominant grasses, several others occur frequently. *Calamovilfa longifolia*, *Andropogon furcatus*, and *A. scoparius* represent the true prairie, while *Aristida longiseta*, *A. fendleri*, *Sitanion hystrix*, and *Distichlis spicata* suggest the short grass plains of the west and southwest.

With the grasses in the association are numerous societies of perennial herbs. The great majority of these are identical with the societies found in the true prairie; others are characteristic of the plains; while a few have invaded the grassland from the chaparral. They constitute an important element in the mixed prairie, and due to them the seasonal aspects of the association show striking variations.

### *Societies*

#### *Vernal aspect:*

<i>Carex brevior</i>	<i>Oxytropis saximontana</i>
<i>Leucocrinum montanum</i>	<i>Euphorbia montana</i>
<i>Calochortus nuttallii</i>	<i>Musineon tenuifolium</i>
<i>Sisyrinchium angustifolium</i>	<i>Oreocarya glomerata</i>
<i>Erysimum asperrimum</i>	<i>Mertensia lanceolata</i>
<i>Erysimum asperum</i>	<i>Lithospermum angustifolium</i>
<i>Sieversia ciliata</i>	<i>Onosmodium occidentalis</i>
<i>Prunus besseyi</i>	<i>Pentstemon albidus</i>
<i>Astragalus caespitosus</i>	<i>Castilleja sessilifolia</i>
<i>Astragalus crassicaarpus</i>	<i>Antennaria aprica</i>
<i>Astragalus gracilis</i>	<i>Achillea lanulosa</i>

*Estival aspect:*

<i>Allium reticulatum</i>	<i>Polygala alba</i>
<i>Yucca glauca</i>	<i>Malvastrum coccineum</i>
<i>Zygadenus intermedius</i>	<i>Opuntia polyacantha</i>
<i>Comandra pallida</i>	<i>Meriolix serrulata</i>
<i>Eriogonum crassifolium</i>	<i>Gaura glabra</i>
<i>Eriogonum multiceps</i>	<i>Lappula occidentalis</i>
<i>Paronychia sessilifolia</i>	<i>Pentstemon gracilis</i>
<i>Drymocallis arguta</i>	<i>Orthocarpus luteus</i>
<i>Astragalus nitidus</i>	<i>Campanula rotundifolia</i>
<i>Oxytropis dispar</i>	<i>Antennaria obovata</i>
<i>Psoralea argophylla</i>	<i>Brauneria angustifolia</i>
<i>Psoralea esculenta</i>	<i>Hymenopappus filifolius</i>
<i>Psoralea tenuiflora</i>	<i>Actinella simplex</i>
<i>Petalostemon oligophyllus</i>	<i>Senecio canus purshianus</i>
<i>Petalostemon purpureus</i>	<i>Senecio fendleri</i>
<i>Linum rigidum</i>	<i>Lygodesmia juncea</i>

*Serotinal aspect:*

<i>Liatris punctata</i>	<i>Aster ptarmicoides</i>
<i>Gutierrezia sarothrae</i>	<i>Artemisia frigida</i>
<i>Chrysopsis foliosa</i>	<i>Artemisia gnaphalodes</i>
<i>Solidago missouriensis</i>	<i>Cirsium megacephalum</i>
<i>Solidago pulcherrima</i>	

The permanence of the mixed prairie depends upon the ability of the tall grasses to maintain themselves successfully. They are apparently able to do this in areas that are not subject to severe grazing; but in other areas, notably along the southern foothills where there has been considerable grazing, the prairie grasses are less abundant, and there is a marked increase in such species as *Artemisia frigida* and *Opuntia polyacantha*. At one point south of Hot Springs there is an overgrazed area which is completely dominated by cactus and sage.

Where the rainfall does not exceed 18 inches, the grassland is able to persist along the tension line with the chaparral, and may

even invade the scrub and the pine savannah to some degree; but where the precipitation exceeds this figure, the grassland gives way to the deciduous forest and the yellow pine. A very slight change in the direction of more moist conditions would result in the extension of the forest area beyond its present limits, while a swing in the other direction would materially reduce the forested zone and permit further extension of the grassland into the Hills.

#### SCRUB CLIMAXES

SAGEBRUSH SUBCLIMAX; ARTEMISIA ASSOCIATION.—The sagebrush association may be regarded as an eastern extension of the



FIG. 11.—Sagebrush association south of Cheyenne River; cultivated fields on right suggest indicator value of sage; secondary succession ruderals seen along roadway.

Basin sagebrush, which makes contact with the mixed prairie along the southern and western foothills, being especially well defined in the former location. The habitat selected as representative of this type is located about 20 miles southwest of Hot Springs, on the lower Edgemont road. After crossing the Cheyenne River the sage occurs much more abundantly, and closely resembles the situation found in the Great Basin (fig. 11).

The dominant species is *Artemisia tridentata*, with *A. filifolia* and *A. cana* as codominants. Other sages in the association are *Artemisia*

*frigida*, *A. gnaphalodes*, *A. caudata*, and *A. canadensis*; but these do not hold an important place in the plant groupings, and are commonly found in the mixed prairie. *Chrysothamnus graveolens*, *Gutierrezia sarothrae*, and *Sarcobatus vermiculatus* are the principal subdominants, the last being abundant in the more saline situations.

The subclimax character of this association is indicated by the presence of many grasses and societies of the mixed prairie, which tend to supersede the sage, except under conditions of overgrazing, as a result of successful competition for water. Where the rainfall is below 16 inches annually the sage assumes a permanent character, which would justify classification of the association as climax rather than subclimax; but the majority of cases indicate that it is subclimax to the mixed prairie.

*Grasses of mixed prairie in sagebrush subclimax*

*Agropyron smithii*

*Bulbilis dactyloides*

*Bouteloua gracilis*

*Sitanion hystrix*

*Societies*

*Tradescantia laramiensis*

*Opuntia fragilis*

*Erysimum asperrimum*

*Gaura glabra*

*Psoralea tenuiflora*

*Pentstemon albidus*

*Petalostemon purpureus*

*Liatris punctata*

*Linum rigidum*

*Lygodesmia juncea*

PETTRAN CHAPARRAL CLIMAX; CERCOCARPUS-RHUS ASSOCIATION. —The chaparral occupies an intermediate zone between the mixed prairie and the montane forest, and is represented in the Black Hills by the *Cercocarpus-Rhus* association. Constituting an extension of the Rocky Mountain chaparral, it occurs chiefly on the western slopes, where it represents the eastern limit of the association. The chaparral is not a continuous zone, being much interrupted by the irregularities of the topography of the region. In places where it is lacking, the grassland makes direct contact with the yellow pine forest.

A good example of this association is located on the South Dakota-Wyoming state line near the L.A.K. ranch, and about 12

miles southeast of Newcastle on Highway 36. At this point there is a typical chaparral dominated by *Cercocarpus parvifolius* and *Rhus trilobata*, with the former occurring in almost pure stands at several stations (fig. 9). *Symphoricarpos racemosus* and *Rosa arkansana* are present, but do not occur as frequently as in the subclimax chaparral of the eastern foothills.

The association is of the open savannah type, and includes many grasses and societies of the mixed prairie as well as societies that are characteristic of the forest. Since the majority of the species are drawn from the climax associations in contact with it, it cannot be considered as having an underflora peculiar to itself. In the open spaces are societies of the prairies, and in the protecting shade of the shrubs are found the forest elements.

#### *Grasses of mixed prairie*

Agropyron riparium	Koeleria cristata
Aristida fendleriana	Oryzopsis hymenoides
Bouteloua curtipendula	Sitanion hystrix
Bouteloua gracilis	Stipa comata

#### *Societies*

Woodsia oregana	Malvastrum coccineum
Juniperus scopulorum	Gaura coccinea
Allium reticulatum	Musineon tenuifolium
Calochortus nuttallii	Oreocarya glomerata
Zygadenus intermedius	Hedeoma hispida
Paronychia sessilifolia	Castilleja sessiliflora
Pulsatilla patens	Campanula rotundifolia
Argemone intermedia	Chrysopsis foliosa
Erysimum asperum	Brauneria angustifolia
Heuchera richardsonii	Hymenopappus filifolius
Oxytropis saximontana	Actinella simplex
Psoralea esculenta	Achillea lanulosa
Petalostemon purpureus	Artemisia frigida
Polygala alba	Cirsium megacephalum
Euphorbia fendleri	

The fact that the societies of this association are in reality invaders from the associations in contact with it, raises the question as

to whether or not it is correct to consider the chaparral as a climax type. Is it not similar in this respect to the sagebrush subclimax? The difference lies in the fact that the sagebrush is actually replaced by the grasses in the succession, while the invader societies in the *Cercocarpus-Rhus* association merely occupy the open spaces between the shrubs, and do not appear to compete successfully with them once they are established. Further, it should be noted that at certain points *Cercocarpus* occurs as a pure stand without any of the underflora previously listed. In view of these facts, and considering that it is clearly a climax type farther west, it seems desirable to classify the Petran chaparral as a relict outpost of a true climax type, which will hold its position in an intermediate climatic zone where the rainfall is approximately 16 inches annually.

CHAPARRAL SUBCLIMAX; QUERCUS-RHUS ASSOCIATION.—The subclimax chaparral occurs as an intermediate zone along the eastern foothills, between the mixed prairie and the montane forest, and also extends eastward along the stream valleys. It occupies the same relative position on the eastern foothills that the *Cercocarpus-Rhus* association holds on the western, but is quite different in its affinities, being definitely related to the marginal type found along the deciduous forest of the east. Like the Petran chaparral, it is not a continuous belt, but occurs chiefly in the lower valleys of the eastern streams and in some instances extends well up into the hills (fig. 12). Studies of this type were made at Belle Fourche, lower Spearfish Canyon, Whitewood, Boulder Canyon, and Hot Springs.

The dominant vegetation is chiefly shrubby, but there are a few trees intermixed which are somewhat dwarfed because of the xerophytic conditions of the habitat.

#### *Dominants*

<i>Corylus rostrata</i>	<i>Prunus melanocarpa</i>
<i>Quercus macrocarpa</i>	<i>Prunus virginiana</i>
<i>Rosa arkansana</i>	<i>Rhus cismontana</i>
<i>Rosa woodsii</i>	<i>Rhus trilobata</i>
<i>Amelanchier alnifolia</i>	<i>Shepherdia argentea</i>
<i>Crataegus sheridana</i>	<i>Cornus stolonifera</i>
<i>Prunus americana</i>	<i>Symphoricarpos occidentalis</i>
<i>Prunus besseyi</i>	<i>Symphoricarpos racemosus</i>

In addition to the bur oak, hawthorn, and plums mentioned, there are several other trees that occasionally are associated with this group, although they properly belong to the deciduous forest. These are *Ulmus americana*, *Fraxinus pennsylvanica*, *Ostrya virginiana*, *Acer negundo*, and *Celtis occidentalis*. As in the case of the mountain chaparral, the societies are derived from the adjacent forest and grassland associations, although some of them reach their highest development in the scrub community. The twofold character of the important societies is indicated in the following lists.

*Woodland or forest societies*

<i>Pteris aquilina</i>	<i>Geranium richardsonii</i>
<i>Filix fragilis</i>	<i>Rhus rydbergii</i>
<i>Disporum trachycarpum</i>	<i>Viola adunca</i>
<i>Unifolium canadense</i>	<i>Viola pubescens</i>
<i>Smilacina sessilifolia</i>	<i>Aralia nudicaulis</i>
<i>Urtica viridis</i>	<i>Sanicula marylandica</i>
<i>Aquilegia canadensis</i>	<i>Washingtonia longistylis</i>
<i>Thalictrum venulosum</i>	<i>Galium aparine</i>
<i>Sanguinaria canadensis</i>	<i>Galium boreale</i>
<i>Vicia americana</i>	<i>Arnica cordifolia</i>
<i>Lathyrus ochroleucus</i>	

*Grassland societies*

<i>Zygadenus elegans</i>	<i>Monarda ramaleyi</i>
<i>Anemone cylindrica</i>	<i>Campanula rotundifolia</i>
<i>Erysimum asperum</i>	<i>Solidago missouriensis</i>
<i>Astragalus carolinianus</i>	<i>Rudbeckia hirta</i>
<i>Psoralea esculenta</i>	<i>Artemisia gnaphalodes</i>
<i>Verbena stricta</i>	<i>Tragopogon porrifolius</i>

Grasses and sedges are not uncommon in this association, species characteristic of the mixed prairie being prominent in the more open phases of the chaparral. In the more mesophytic locations, where the shrubs grow taller and closer together, the meadow species *Phleum pratense*, *Poa palustris*, and *Agrostis palustris* occur.

The subclimax character of this association is suggested by its heterogeneous floristic composition and by the variability of its

aspects. Unlike the *Cercocarpus-Rhus* association, it has no single dominant or group of dominants, varying widely in this respect in the different habitats observed. Further, it apparently lacks the stability of a climax association, and appears to be going over to grassland on the one side and coniferous forest on the other. It suggests a relict vegetation of a wetter climatic cycle which is gradually losing ground under present conditions.



FIG. 12.—Stand of *Quercus macrocarpa* at mouth of Boulder Canyon near Sturgis, representing intermediate zone between grassland and montane forest.

#### FOREST CLIMAXES

DECIDUOUS FOREST POSTCLIMAX; QUERCUS ASSOCIATION.—The deciduous forest is very poorly represented in the Black Hills, and is undoubtedly a remnant of a once more extensive oak-hickory association of which but a few dominants remain. The probable history of this association has already been discussed, but the successional relationships require a brief statement.

The trees that make up this association are the same as occur as dwarf forms in the subclimax chaparral; and there would be no justification for including this association in the present analysis were it not for the fact that, at a few points, they attain treelike dimensions. In several places, notably at Whitewood, Dark Canyon, and Spearfish, the oak becomes a rather large tree of forest propor-



tions, and it would be inaccurate to classify it and the associated trees as subclimax chaparral. *Quercus macrocarpa* is the sole dominant; but with it are several other trees of subdominant rank, *Ulmus americana*, *Fraxinus pennsylvanica*, *Acer negundo*, and *Celtis occidentalis*.

The societies are not essentially different from those noted for the subclimax chaparral, except for the greater number of mesophytic herbs and fewer species characteristic of the grassland. In fact there is no sharp line of demarcation between the oak chaparral and the oak forest, the latter being merely the response of the same species to more favorable conditions. For this reason, the oak association is considered as postclimax, since it is an expression in terms of limited growth and development of what was once a much more widespread condition. Locally in the more favorable habitats, the association is undoubtedly climax rather than postclimax, as it is maintaining itself successfully and even slightly extending its range. In general, however, it is a relict type which is passing back through the chaparral stage to the grassland on the one hand, and being replaced by the more xerophytic yellow pine on the other.

PETTRAN MONTANE FOREST CLIMAX; *PINUS SCOPULORUM* ASSOCIATION.—The montane forest is the outstanding association in the Black Hills, dominating the central areas and extending projecting arms down the rocky ridges of the foothills, where it makes contact with the chaparral and the mixed prairie (fig. 10). The forested region lies mainly within the limits of the Harney and Black Hills National Forests and Custer State Park, which have a combined area of approximately 1,435,000 acres. In addition to this there is a considerable acreage that is privately controlled by mining companies and other interests. Not all of this area is timbered, however, as one of the striking features of the forest is the occurrence of numerous open parks, meadows, and prairies, which in some instances are very extensive.

This eastern outlier of the great coniferous forests of the Rocky Mountains is dominated by *Pinus scopulorum*, which grows in almost pure stands, differing in this respect from the yellow pine forests of the West, where it often is associated as a dominant with *Pseudotsuga mucronata*, *Abies concolor*, and *Pinus murrayana* (5).

*Picea albertiana* occurs as a subdominant in the Harney region and on the more moist slopes in the northern valleys; *Pinus murrayana* occurs in but one locality, forming a subclimax association in the vicinity of Nahant; and there is a small society of *Pinus flexilis*, numbering about forty mature trees, in the Needles on the boundary line between Pennington and Custer counties.

*Pinus scopulorum* is the most xerophytic of the dominants of the montane forest, which doubtless accounts for its extension beyond the range of the other members of this formation. In Arizona and New Mexico, WOOLSEY (32) finds that "no stand of large extent is likely where the annual rainfall is less than 20 inches." Scattering stands do occur in that region on northerly exposures with as little as 16.6 inches. This agrees essentially with the situation in the Black Hills, where the best stands of yellow pine are found in the central and northern regions, in which the average annual rainfall ranges from 21.34 to 24.45 inches. In the drier habitats at Hot Springs and Belle Fourche, where the rainfall is 18.41 and 15.03 respectively, the yellow pine grows as an open savannah and fails to develop good mature stands.

The savannah represents the first of three rather well defined aspects of the yellow pine association, and occurs near the contact zones with the chaparral, where the rainfall is 15-18 inches. In this phase the trees are widely scattered in discontinuous clumps, and the intervening spaces are occupied by grassland of the mixed prairie type. There is little evidence of reproduction, and the pines are stunted and lacking in vigor.

The second aspect is the open park. This is the most common type in the Hills, and occurs in situations where the rainfall is 20-22 inches. Here the pines grow to full maturity, attaining a height of 75 feet or more, reproduction is adequate, and the light factor is favorable for sapling development. With the yellow pine in this phase, there is usually a scattering of *Picea albertiana*, *Populus tremuloides*, and *Betula papyrifera*, the last two being related to a secondary stage in the succession.

In addition to these there is an understory of shrubs, which are in part species characteristic of the chaparral and the deciduous forest associations.

*Shrub societies*

Juniperus sibirica	Rubus strigosus
Corylus rostrata	Rosa arkansana
Ostrya virginiana	Amelanchier alnifolia
Berberis aquifolium	Ceanothus sanguineus
Ribes cereum	Cornus stolonifera
Ribes inerme	Arctostaphylos uva-ursi
Ribes setosum	Sambucus racemosa
Physocarpus intermedius	Viburnum pauciflorum
Spiraea lucida	Symphoricarpos racemosus
Rubus americanus	Lonicera glaucescens

The third aspect is the closed forest. This is more mesophytic than either of the preceding, with a rainfall approximating 24 inches. It is found in the northern hills, but the number of mature stands is limited because of the extensive use of the pine for mining purposes, severe losses by fire, and *Dendroctonus* beetles. The second growth stands are developing into this closed type, and density of stand rather than reproduction is one of the practical problems confronting the forester.

Shrubs are less abundant in the closed type, and are replaced by numerous herbaceous societies. There is no sharp line separating the open park from the closed forest, so that the societies listed are in general characteristic of both phases. The seasonal aspects are best expressed by grouping into two rather than three divisions, as the serotinal aspect is not clearly differentiated. It should be noted that many of the spring-blooming species continue on well into the summer.

*Herbaceous societies**Vernal aspect:*

Filix fragilis	Thermopsis rhombifolia
Woodsia oregana	Vicia americana
Oryzopsis asperifolia	Lathyrus ochroleucus
Unifolium canadense	Polygala senega
Disporum trachycarpum	Viola adunca
Clematis pseudoalpina tenuiloba	Viola canadensis
Actaea rubra	Aralia nudicaulis

*Cornus canadensis*  
*Mertensia lanceolata*  
*Galium boreale*

*Valeriana ceratophylla*  
*Antennaria howellii*  
*Antennaria parvifolia*

*Estival aspect:*

*Lilium montanum*  
*Smilacina sessilifolia*  
*Limnorchis hyperborea*  
*Peramium repens*  
*Corallorrhiza multiflora*  
*Thalictrum venulosum*  
*Aconitum ramosum*  
*Geranium richardsonii*  
*Epilobium adenocaulon*  
*Sanicula marylandica*  
*Zizia cordata*  
*Pyrola minor*  
*Pyrola secunda*

*Pterospora andromeda*  
*Frasera speciosa*  
*Castilleja sulphurea*  
*Linnaea americana*  
*Valeriana occidentalis*  
*Aster laevis*  
*Aster lindleyanus*  
*Aster ptarmicoides*  
*Erigeron glabellus*  
*Anaphalis subalpina*  
*Arnica rydbergii*  
*Senecio canus purshianus*  
*Senecio flavulus*

The relation between forest and grassland, where they make contact along the margins of the meadows and prairies, is difficult to interpret. Apparently the two formations are in equilibrium, as there is no evidence that either is extending its range at the present time. The occurrence of parks and prairies, within the main limits of the yellow pine association, is a characteristic that has been observed wherever this association is found; and there are many examples of this throughout the Hills, especially at Reynolds Prairie and in the Limestone District.

The explanation of this situation is probably tied up with the influence of climatic and edaphic factors on yellow pine reproduction. PEARSON (20) has made extensive studies of the reproduction of the yellow pine in the southwest, and finds that "reproduction is better on the coarser, sandy, gravelly, or stony soils than on the finer soils regardless of geologic origin." The meadows and prairies have a soil of very fine texture, and the softness and depth of this surface material explain in part the absence of trees. It is possible that the absence of trees in the Red Valley is due to this same factor, as they occur on the Hogback Ridge outside of the valley where the climatic

conditions are the same. Once the tree has established itself, its soil requirements are not sharply delimited; but it does best on the limestone formations, where the largest trees and the finest stands are to be found.

The protection afforded the seedlings by the mature trees is an important factor. The effect of this cover is to reduce air movement, with a consequent decrease in transpiration, and to moderate the temperature, thus reducing the amount of winter killing in the seedling crop. On the other hand, the yellow pine is intolerant of shade except in the early seedling stage, and saplings coming up under mature stands will not reach maturity. The best reproduction is obtained in the open park, where a reasonable degree of cover protection is provided, with sufficient light to meet later growth requirements.

The fact that the pine grows on the Hogback Ridge, on the exposed ridges of the foothills, and on such outlying extrusions as Bear Butte suggests that the edaphic factor is the limiting one rather than the climatic factors, where the average annual rainfall exceeds 18 inches. Below that figure successful competition is impossible, and the forest is replaced by chaparral and grassland.

**BOREAL FOREST POSTCLIMAX; PICEA ALBERTIANA ASSOCIATION.**—Although the white spruce is commonly found in the yellow pine association, it does not properly belong to the climax montane forest, but should be considered as a straggler from the boreal forest. This is indicated by the fact that the spruce dominates only in the colder situations, where the factors of moisture, exposure, and temperature approximate those of its main range. This is well illustrated in upper Spearfish Canyon, where the spruce replaces the yellow pine (fig. 7). At Savoy the two trees occur in equal numbers, but beyond Cheyenne Crossing and upstream for several miles, the north-facing slopes are covered by a stand in which the spruce is the dominant tree.

This type of association is not widespread, but it is sufficiently definite, where it does occur, to warrant separate treatment. In view of the probable vegetational history of the spruce, it seems proper to regard the association as a postclimax relict of a once more widespread boreal forest. The societies found are practically identical with those of the yellow pine association, but there are a few additional species that should be noted.

*Societies*

Botrychium lanceolatum	Geranium viscosissimum
Carex peckii	Viola pubescens
Cypripedium parviflorum	Viola renifolia
Calypso bulbosa	Circaea alpina
Peramium repens	Moneses uniflora
Aquilegia brevistyla	Dodecatheon pauciflorum
Fragaria americana	Mimulus langsdorffii

SECONDARY SUCCESSION SUBCLIMAX; PINUS MURRAYANA ASSOCIATION.—The lodgepole pine is not abundant in the Black Hills, and occurs in sufficient numbers to constitute an association only in the vicinity of Nahant. Isolated specimens have been reported as occurring on the old road between Rapid City and Hill City, near the Hardy Ranger Station, and south of Custer Peak. The habitat studied lies just west of the Chicago, Burlington and Quincy tracks on the north fork of Rapid Creek, about three quarters of a mile south of the Bulldog Ranch.

The association is a typical secondary succession community, and closely resembles the lodgepole burn forests of Colorado described by CLEMENTS (3). It represents a subclimax stage to the yellow pine climax, which is gradually replacing it. At the present time there are areas where about 75 per cent of the trees are lodgepole pine, but in the major portion of the region the yellow pine is codominant with the lodgepole, and in some places has become the dominant tree. The secondary character of the association is indicated by the societies, which include many plants commonly found in the burn forest. Chief among these are *Populus tremuloides*, *Betula papyrifera*, *Vaccinium scoparium*, *Chamaenerion angustifolium*, *Sedum stenopetalum*, *Anaphalis subalpina*, *Rudbeckia hirta*, *Arctostaphylos uva-ursi*, and *Drymocallis arguta*.

SECONDARY SUCCESSION SUBCLIMAX; BETULA-POPULUS ASSOCIATION.—This association represents another aspect of the secondary succession which follows burning or clear cutting. Where the lodgepole is present, the birch-aspen association constitutes a preclimax stage leading up to the lodgepole subclimax; but, in the absence of the lodgepole, it becomes a subclimax stage which directly precedes the yellow pine climax (fig. 13).

A good example of this association is found on the slopes of Terry Peak, above the town of Trojan, where fire and mining operations have resulted in the destruction of the original stand of yellow pine. *Populus tremuloides* and *Betula papyrifera* are the dominant trees, with which are *Prunus melanocarpa*, *P. americana*, *P. pennsylvanica*, and *Sorbus scopulina*. The presence of young pines and



FIG. 13.—Secondary succession of birch and aspen near Cheyenne Crossing; scattering specimens of pine and spruce seen throughout association.

spruces indicates the direction of the succession toward the montane forest climax. The societies in this association, like the preceding one, include many species that are characteristic of the secondary succession.

#### *Societies*

<i>Pteris aquilina</i>	<i>Geranium richardsonii</i>
<i>Juniperus sibirica</i>	<i>Chamaenerion angustifolium</i>
<i>Oryzopsis asperifolia</i>	<i>Aralia nudicaulis</i>
<i>Clematis pseudoalpina tenuiloba</i>	<i>Arctostaphylos uva-ursi</i>
<i>Sedum stenopetalum</i>	<i>Vaccinium scoparium</i>
<i>Rubus strigosus</i>	<i>Castilleja sulphurea</i>
<i>Fragaria americana</i>	

## II. Preclimax or developmental stages

### HYDRIC SERIES

POND STAGE.—Although the region is characteristically dry and inclines toward the xeric side in its climax stages, there are numerous small ponds and water holes that persist throughout the summer, except in seasons of extreme dryness. They have a characteristic hydric flora, including many cosmopolitan species which occur in several different groupings. Thus, in a pond between Fairburn

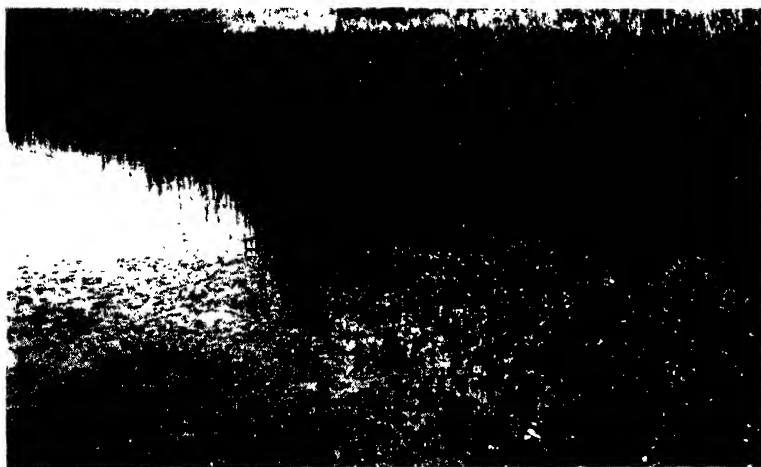


FIG. 14.—*Marsilea vestita* and *Eleocharis palustris* in a pond association between Fairburn and Buffalo Gap.

and Buffalo Gap, there is a *Marsilea vestita*-*Eleocharis palustris* combination, the former occupying the inner zone and the latter dominating the outer (fig. 14). With the latter are *Eleocharis acicularis*, *Lophocarpus depauperatus*, and *Gratiola virginiana*. These are replaced by *Carex brevior* and various prairie grasses along the outer margin. In a pond near Hot Springs, the grouping is *Eleocharis glaucescens*, *Juncus torreyi*, and *Roripa nasturtium-aquaticum*. At Beulah, in a water hole and swale, the grouping is *Eleocharis glaucescens*, *Scirpus americanus*, *Juncus ater*, and *Carex praegracilis*. Other combinations include *Sagittaria arifolia*-*Herpestes rotundifolia* found south of Rapid City; *Eleocharis glaucescens*-*Polygonum amphibium*-*Heteranthera limosa* near Wind Cave; and *Typha latifolia* at Aztec.



Sylvan Lake in the Harney region is the only lake in the Hills. It is not a natural one, but has been formed by the construction of a dam at its west end, which impounds the headwaters of the south fork of Spring Creek. It is bounded by towering rocks on three sides, but its eastern margin is low and swampy. At this point there is a typical *Typha-Juncus-Scirpus* association. The cattail is the rarer *Typha angustifolia*, with which are *Juncus brevior*, *Scirpus atrocinctus*, *Carex rostrata*, *C. scoparia*, *Panicularia grandis*, *Ranunculus pennsylvanicus*, *Mentha glabrior*, and *Scutellaria galericulata*.

STREAMSIDE STAGE; BETULA-SALIX ASSOCIATION.—The numerous streams that arise in the Limestone District, flow outward to the Red Valley, and thence to the Cheyenne and Belle Fourche rivers, have a vegetation characterized by shrubby dominants that are principally members of two genera, *Salix* and *Betula*. *Populus* is well represented at the lower limits of the streams, but gradually disappears upstream.

Studies of the streamside were made at four points, Castle Creek above Deerfield and Boxelder Creek above Nemo, representing the upper phase of the association; and Spearfish Creek and Hot Brook Creek, illustrating the successional relationships at the lower limits.

The upper reaches of these mountain streams are characterized by a dense growth of shrubs that occupies the margin of the stream and completely screens it from view. The most common dominants are *Betula fontinalis*, *Salix bebbiana*, *S. scouleriana*, and *S. fendleriana*. Other shrubby forms which are found sparingly intermixed with the four dominants are *Salix fluviatilis*, *S. exigua*, *Cornus stolonifera*, *C. baileyi*, *Prunus melanocarpa*, *Corylus rostrata*, *Amelanchier alnifolia*, *A. oreophila*, *Rosa woodsii*, *Ribes inerme*, and *Rubus americanus*. The herbaceous flora consists of the hydrophytic societies that grow in the water or along the water's edge, and the mesophytic societies that grow on the banks under the shade of the dominant shrubs.

#### *Hydrophytic societies*

Equisetum arvense	Triglochin palustris
Equisetum laevigatum	Alopecurus aequalis
Potamogeton heterophyllus	Calamagrostis canadensis
Potamogeton pectinatus	Panicularia nervata

<i>Scirpus microcarpus</i>	<i>Juncus ensifolius</i>
<i>Eleocharis glaucescens</i>	<i>Juncus saximontanus</i>
<i>Carex aurea</i>	<i>Batrachium trichophyllum</i>
<i>Carex disperma</i>	<i>Ranunculus cymbalaria</i>
<i>Carex lanuginosa</i>	<i>Callitriche palustris</i>
<i>Carex nebraskensis</i>	<i>Steironema ciliatum</i>
<i>Carex rostrata</i>	<i>Veronica americana</i>
<i>Carex stipata</i>	<i>Limosella aquatica</i>

*Mesophytic societies*

<i>Limnorchis hyperborea</i>	<i>Moneses uniflora</i>
<i>Actaea arguta</i>	<i>Gentiana plebeja</i>
<i>Ranunculus macounii</i>	<i>Gentiana strictiflora</i>
<i>Heuchera richardsonii</i>	<i>Prunella vulgaris</i>
<i>Vicia americana</i>	<i>Stachys palustris</i>
<i>Viola canadensis</i>	<i>Castilleja sulphurea</i>
<i>Sanicula marylandica</i>	<i>Galium triflorum</i>
<i>Zizia cordata</i>	<i>Linnaea americana</i>
<i>Pyrola asarifolia</i>	

The dominant birches and willows of the upper streamside are partially replaced in the lower canyons by *Populus angustifolia*, *P. acuminata*, and *P. sargentii*. *Salix bebbiana* and *S. fendleriana* persist, and with them are found *S. balsamifera*, *S. fluviatilis*, and *S. lutea*. Intermixed with these are oak, elm, boxelder, ash, and plum, suggesting a gradual transition in the direction of the deciduous forest association.

The successional relationships of the streamside association can be interpreted variously. The dominants in the association belong to a developmental rather than to a climax stage; but there is a permanence to this type of plant community which might be considered as an indication of a climax or subclimax situation. A clue is afforded, however, in the occurrence of several of these dominants in an intermediate zone between the upland meadow and the montane forest (fig. 15). This indicates the true successional position as pre-climax to the montane forest, at least in the upper stream phase. In the lower canyons the *Betula-Salix-Populus* association may be

considered as a subclimax, which under very favorable conditions will be replaced by the deciduous forest.

**UPLAND MEADOW STAGE; GRASS-SEDGE ASSOCIATION.**—The finest examples of the upland meadow are located in the northwest portion of the Limestone District. Here they reach their highest development, and exhibit the most luxuriant growth of meadow grasses and herbs to be found anywhere in the Hills. The present studies were



FIG. 15.—Meadow in Limestone District showing good zonation: marginal shrub *Salix bebbiana*; intermediate zone made up of *Populus tremuloides*; *Pinus scopulorum* in background.

made at three stations: (1) one mile east of the Hardy Ranger Station; (2) in the vicinity of Crooks Tower, 5 miles south of Mountain View Ranch; and (3) at the head of Castle Creek, 12 miles west of Deerfield.

The plateau is mesophytic, having an average annual rainfall of 21–25 inches. It has an elevation of 6500–7000 feet, and in consequence the snow remains in the meadow depressions late in the spring. These two factors operate together to supply sufficient moisture for the development of an abundant meadow flora. This reaches its most brilliant aspect in late June and early July, when the meadows are very colorful.

The meadow dominants are mainly grasses and sedges, with *Poa* predominating, but there are no outstanding groupings.

### *Dominants*

<i>Torresia odorata</i>	<i>Poa pratensis</i>
<i>Stipa richardsonii</i>	<i>Bromus marginatus</i>
<i>Oryzopsis asperifolia</i>	<i>Bromus porteri</i>
<i>Phleum pratense</i>	<i>Agropyron repens</i>
<i>Agrostis hiemalis</i>	<i>Elymus innovatus</i>
<i>Danthonia intermedia</i>	<i>Carex festivella</i>
<i>Poa compressa</i>	<i>Carex hoodii</i>
<i>Poa fendleriana</i>	<i>Carex tenera</i>
<i>Poa interior</i>	

### *Societies*

<i>Juncoides comosum</i>	<i>Lupinus sericeus</i>
<i>Juncus confusus</i>	<i>Geranium viscosissimum</i>
<i>Iris missouriensis</i>	<i>Viola adunca</i>
<i>Sisyrinchium angustifolium</i>	<i>Viola canadensis</i>
<i>Delphinium bicolor</i>	<i>Dodecatheon pauciflorum</i>
<i>Aconitum ramosum</i>	<i>Valeriana ceratophylla</i>
<i>Anemone globosa</i>	<i>Antennaria rosea</i>
<i>Ranunculus cardiophyllus</i>	<i>Rudbeckia hirta</i>
<i>Thalictrum venulosum</i>	<i>Balsamorhiza sagittata</i>
<i>Sieversia ciliata</i>	<i>Helianthella quinquinervis</i>
<i>Astragalus alpinus</i>	<i>Gaillardia aristata</i>
<i>Oxytropis gracilis</i>	<i>Crepis riparia</i>

The marginal zonation of the meadows is very clearly defined, and indicates the probable relationship of the meadow in the succession (fig. 15). The first zone beyond the meadow proper, when present, consists of low-growing shrubs: *Ceanothus velutinus*, *Dasiphora fruticosa*, and *Juniperus sibirica*. This is followed by a zone of willows, the principal species being *Salix bebbiana* and *S. scouleriana*. The outermost zone is made up of *Populus tremuloides*, which in turn makes contact with the climax yellow pine forest.

The meadow is thus a developmental phase leading up to the climax montane forest through the willow and aspen stages. Its

persistence can be attributed to edaphic factors and to the topography of the habitat. The former refer to the physical characteristics of the soil, which are unfavorable to the establishment of tree seedlings, as in the case of the prairie habitats previously discussed. The meadows invariably occupy basin-like depressions in which the snow accumulates and remains late in the spring. This condition favors the grasses and herbs, and makes invasion of the meadow by the forest an extremely slow process. Another restricting factor is the effect of continued grazing and haying, which prevents seedling development. The analysis of the causes for the permanency of the meadow community, which indicates that the factors at work are other than climatic, is the basis for the present classification of the upland meadow as a preclimax stage.

#### XERIC SERIES

The early stages in the xeric succession are of three general types: (1) the rock pioneer, (2) the erosion pioneer, and (3) the secondary succession pioneer. The first is well illustrated by the plant communities found on the exposed summits of the mountain peaks, and on the rocky walls of many of the canyons. The second can be studied where the original vegetational cover has been destroyed by the erosive action of water and wind. The third is similar in aspect to the second, but is the result of different factors which are largely humanistic. These include overgrazing, road-building, and attempts at agriculture which are later abandoned.

**ROCK PIONEER STAGE.**—Studies of this stage were made at three points: (1) Harney Peak, elevation 7240 feet; (2) Terry Peak, elevation 7071 feet; and (3) Custer Peak, elevation 6794 feet. In each instance the summit is a rocky windswept habitat, where the climatic and edaphic factors are unfavorable to any but the hardiest pioneer plants. Rock lichens and mosses represent the earliest stages in the succession, common forms being *Placodium elegans*, *Rhizocarpon geographicum*, and *Grimmia*. Grasses and herbaceous crevice plants follow the lichen-moss stage, and are in turn replaced by crevice shrubs. These are followed by the subclimax birch and aspen, and ultimately the climax pine-spruce association is established.

*Herbaceous crevice plants*

<i>Filix fragilis</i>	<i>Draba luteola</i>
<i>Woodsia scopulina</i>	<i>Arabis fendleri</i>
<i>Selaginella densa</i>	<i>Sedum stenopetalum</i>
<i>Calamagrostis purpurascens</i>	<i>Drymocallis fissa</i>
<i>Danthonia spicata</i>	<i>Drymocallis pseudorupestris</i>
<i>Carex festivella</i>	<i>Androsace diffusa</i>
<i>Carex siccata</i>	<i>Myosotis alpestris</i>
<i>Cerastium oreophilum</i>	<i>Antennaria rosea</i>
<i>Sagina saginoides</i>	

*Crevice shrubs*

<i>Juniperus sibirica</i>	<i>Physocarpus monogynus</i>
<i>Ribes cereum</i>	<i>Rubus strigosus</i>
<i>Ribes irriguum</i>	<i>Dasiophora fruticosa</i>
<i>Ribes parvulum</i>	

Crevice plants found at lower elevations in the canyons include:

<i>Polypodium hesperium</i>	<i>Parnassia parviflora</i>
<i>Cheilanthes feei</i>	<i>Heuchera hispida</i>
<i>Pellaea glabella</i>	<i>Heuchera richardsonii</i>
<i>Woodsia oregana</i>	<i>Tellima parviflora</i>
<i>Aquilegia brevistyla</i>	<i>Petrophyton caespitosum</i>
<i>Aquilegia canadensis</i>	<i>Musineon tenuifolium</i>
<i>Sophia pinnata</i>	<i>Antennaria aprica</i>

EROSION PIONEER STAGE.—These stages are locally common wherever the work of water and wind has opened up a new habitat to invasion; but the most striking examples are located east of the Hills proper, in the White River Bad Lands, where the eroded surfaces of the Great Wall exhibit the first evidences of the xeric succession. Early pioneers in such habitats are:

<i>Allium reticulatum</i>	<i>Lappula occidentalis</i>
<i>Eriogonum crassifolium</i>	<i>Oreocarya glomerata</i>
<i>Eriogonum multiceps</i>	<i>Gutierrezia sarothrae</i>
<i>Monolepis nuttalliana</i>	<i>Grindelia squarrosa</i>
<i>Atriplex canescens</i>	<i>Chrysothamnus graveolens</i>
<i>Lesquerella alpina argillosa</i>	<i>Chrysothamnus wyomingensis</i>
<i>Pachylophus caespitosus</i>	

These are followed by grasses of the mixed prairie, *Bulbils*, *Koeleria*, *Sitanion*, and *Stipa* in the later stages of the succession.

SECONDARY SUCCESSION PIONEER STAGE.—In this stage, the first vegetation consists of ruderals. There are many situations illustrating this type of community, but one of the most clearly defined habitats occurs where the original cover has been destroyed by recent highway construction. The practice in widening the highways is to plow up a zone considerably wider than the actual highway, from which the top soil is scraped for grading purposes (fig. 11). This leaves an exposed strip on either side which is soon invaded by a characteristic group of annual plants.

### *Secondary succession annuals*

Echinochloa crus-galli	Capsella bursa-pastoris
Panicum barbipulvinatum	Camelina sativa
Cenchrus pauciflorus	Cleome serrulata
Festuca octoflora	Medicago lupulina
Bromus japonicus	Euphorbia marginata
Bromus tectorum	Malva rotundifolia
Chenopodium album	Verbena bracteosa
Chenopodium paganum	Solanum rostratum
Salsola pestifer	Plantago purshii
Amaranthus retroflexus	Ambrosia elatior
Lepidium apetalum	Dysodia papposa

These annuals are followed by deep-rooted perennials such as *Gutierrezia sarothrae*, *Yucca glauca*, *Malvastrum coccineum*, *Grindelia perennis*, *Artemisia frigida*, *Brauneria angustifolia*, *Ratibida columnaris*, *Petalostemon purpureus*, and *Eriogonum multiceps*. The perennials form an open cover, and the entry of the grasses of the mixed prairie follows, completing the succession.

The studies in the Black Hills were pursued under the direction of Dr. HENRY C. COWLES, whose constant encouragement and helpful criticism have been invaluable in the preparation of this paper. Dr. F. E. CLEMENTS very kindly supplied me with advance copies of his new list of the climax formations and associations of North America, and also criticized my analysis of the successional relation-

ships in the Hills. In connection with the work in the field, I am indebted to Professor A. C. McINTOSH for many courtesies, including the unlimited use of his laboratory at the State School of Mines at Rapid City. I desire especially to express my thanks to Dr. AVEN NELSON and Dr. P. A. RYDBERG, who determined the major portion of the specimens collected. Thanks are also due to Professor A. S. HITCHCOCK and Mrs. AGNES CHASE, who determined the grasses; and to Mr. KENNETH K. MACKENZIE, who examined the sedges.

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# RHIZOCTONIA DISEASE ON CERTAIN AQUATIC PLANTS<sup>1</sup>

W. S. BOURN AND BERNICE JENKINS

(PLATES XI-XIV AND SIX FIGURES)

## Introduction

The inland waters of Back Bay, Virginia, and Currituck Sound, North Carolina (fig. 1) have long been known to represent one of the most important winter feeding grounds for migratory wild fowl in the United States. Here aquatic duck-food plants, such as *Potamogeton pectinatus* L., *P. perfoliatus* L., *Ruppia maritima* L., *Vallisneria spiralis* L., and *Najas flexilis* (Willd.) Rostk. & Schmidt formerly thrived in great abundance. In 1918, almost simultaneously with the opening and enlargement of the Albemarle and Chesapeake Canal, however, these plants began to die out, and by the end of 1926 vast areas were practically denuded of their aquatic vegetation. This destruction of the plant life has wrought an enormous economic loss upon thousands of the native population, who derived their living from gunning and fishing. Shooting clubs and sportsmen have practically deserted their large investments in the region, since wild ducks and geese in any appreciable numbers are no longer attracted there. At the request of WILLIAM E. COREY, a prominent sportsman of New York, who has been interested in these waters, the Boyce Thompson Institute for Plant Research in 1925 undertook a study of the causes for the disappearance of the aquatic duck-food plants, and early the following year the writers were assigned to the investigation.

The region involved represents an area of approximately 300 square miles of inland waters. At 26 widely scattered stations in this area, periodic analyses have been made to determine the salinity of the water. Field observations have been made almost daily from March to October. Plant specimens have been collected for study

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

at various stages of growth and decay. Experiments to determine the influence of soil, water, and salinity upon the growth and the disease of aquatic plants native to the region have been carried on for more than two years in the greenhouse. Inoculation experiments,

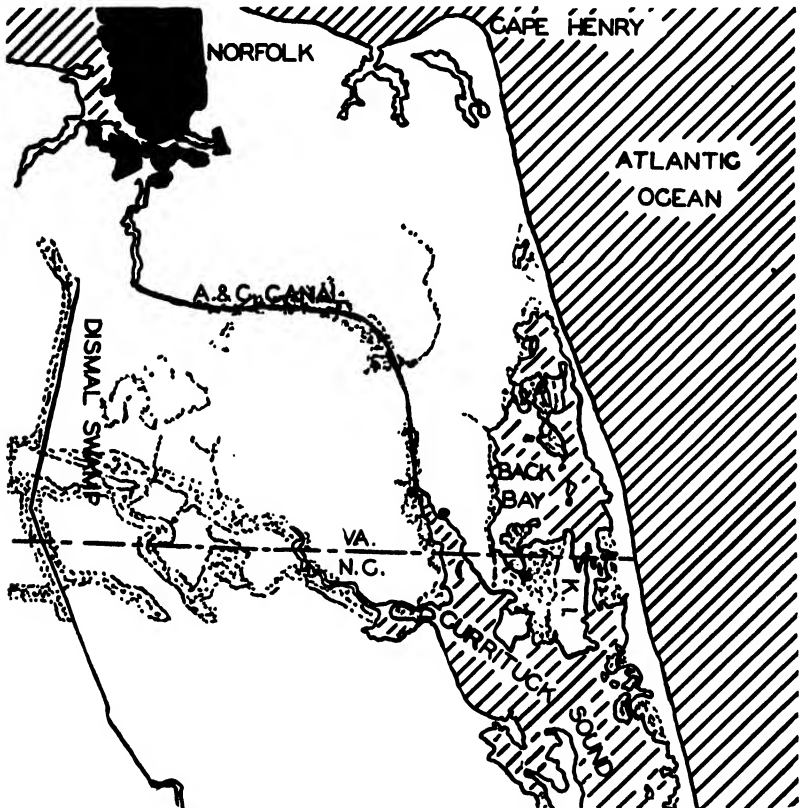


FIG. 1.—Map of Back Bay, Virginia, and Currituck Sound, North Carolina, regions.

using cultures of a fungus isolated from diseased aquatic plants and from diseased potato plants, have extended over the same period of time.

While other injurious factors have been encountered during the course of the investigations, it is the purpose of this paper to describe and discuss a *Rhizoctonia* disease which we believe to be the

factor of greatest importance, and the one most directly responsible for the disappearance of the aquatic duck-food plants in Back Bay and Currituck Sound.

The fungus *Rhizoctonia solani* Kühn is widely distributed in America and elsewhere, having been reported from most regions of the world where the potato is a staple crop. In addition to the potato, which appears to be the most common host, the host plants of this fungus represent many families of dicotyledons and a number of monocotyledons. PELTIER (4) lists about 165 species of plants susceptible to *R. solani*. PALO (3) has recently reported this fungus causing a serious disease of rice in the Philippines. Regardless of the multitude of host plants reported in the literature, which we shall not attempt to review, the occurrence of *Rhizoctonia* on rice plants, to our knowledge, is the closest approach to an occurrence on aquatic plants thus far reported. When we consider such a wide range of hosts, and the fact of its occurrence on semi-aquatic plants, the discovery of *R. solani* in a purely aquatic situation is less surprising, if unusual.

L. O. KUNKEL, pathologist of the Boyce Thompson Institute for Plant Research, first isolated pure cultures of *Rhizoctonia solani* from diseased aquatic plants collected from Back Bay, Virginia, in September 1925. Since that time we have repeatedly isolated pure cultures of this fungus from diseased plants collected from the infected area. We have inoculated plants growing in the greenhouse aquaria with sclerotia from these cultures. These inoculated plants became infected almost immediately, and later pure cultures of the fungus were re-isolated from them. We have determined that this aquatic strain of *Rhizoctonia* will not only kill aquatic plants but will also attack such terrestrial plants as the potato; furthermore, that a strain of *R. solani*, isolated from diseased potato plants, will attack aquatic plants.

#### Occurrence and identification of *Rhizoctonia* on diseased and dead tissue

In the field it was noticed that signs of *Rhizoctonia* infection first became visible as dark lesions on portions of the plant stems, near the first node at the surface of the soil. Infection took place on this

portion of the plant regardless of the depth of the water in which the plant was growing. By taking one-fourth inch segments of these diseased stems, washing them half a dozen times in several cubic centimeters of sterile distilled water, to remove water bacteria, etc., and then transferring these segments to potato and to green string bean agar, pure cultures of *R. solani* were obtained. After the segments had been in the agar one to two days, the fungus could be observed growing out into the agar from the cut ends of the segments. At the end of the fourth day the growth was about the size of a half-dollar, and very shortly thereafter the entire plate became covered by the mycelial growth. About the second week small white spots, 1-2 mm. in diameter, appeared in the older mycelia near the center of the agar plates. Within a few more days these white spots became the black sclerotia shown in pl. XI. Concentric zonations are characteristic of these cultures, and showed plainly in most of them. In measurements of hyphal and sclerotial cells, in mycelial and cultural characteristics, and in physical appearances this fungus corresponds closely to the descriptions of *R. solani* given in the literature. The aquatic strain and the potato strain of *Rhizoctonia* are compared in the cultures shown in pl. XI. *A* and *B* are cultures of the fungus isolated in 1926 from diseased aquatic plants collected from Back Bay, on potato agar and on green string bean agar respectively; *C* and *D* are cultures on potato and green string bean agar respectively of the aquatic strain of *Rhizoctonia* re-isolated in 1927 from greenhouse aquatic plants used in the inoculation experiments; *E* and *F* represent cultures of *R. solani* isolated from diseased potato plants, on potato and on green string bean agar respectively.

**Inoculations with *Rhizoctonia*; production of the disease;  
subsequent re-isolation of the fungus**

To determine the effects of *Rhizoctonia* inoculations upon five species of aquatic duck-food plants, *Potamogeton pectinatus*, *P. perfoliatus*, *Ruppia maritima*, *Vallisneria spiralis*, and *Najas flexilis*, experiments were set up in the greenhouse aquaria. In these experiments four types of soil were used. Water solutions were made up by diluting boiled Long Island Sound water with distilled water

until the solutions contained a saline content equal to the average for Back Bay, to one-half, and to double that concentration. Ten gallons of water was used in each culture. This furnished two feet of water over six inches of soil in the vessels. The form of the plants used in these cultures was either seeds, tubers, or young plants. Inoculations were made by placing in the soil sclerotia of the strain of *Rhizoctonia* isolated from Back Bay aquatic duck-food plants. The results obtained for the inoculation cultures, using *Potamogeton pectinatus*, are given in table I.

TABLE I

RESULT OF INOCULATION OF POTAMOGETON PECTINATUS WITH AQUATIC STRAIN OF RHIZOCTONIA SOLANI

SOIL	SALINITY OF WATER IN PERCENTAGE OF NORMAL SEA WATER	AVERAGE LENGTH OF PLANT LIFE	
		Inoculated	Check
Sterile quartz sand . . . . .	3.5	45 days	100 days
Sterile quartz sand . . . . .	7.0	20 days	100 days
Sterile quartz sand . . . . .	14.0	35 days	90 days
Sterile Back Bay soil . . . . .	3.5	No growth*	No growth*
Sterile Back Bay soil . . . . .	7.0	No growth*	No growth*
Sterile Back Bay soil . . . . .	14.0	No growth*	No growth*
Untreated Back Bay soil . . . . .	3.5	40 days	200 days
Untreated Back Bay soil . . . . .	7.0	25 days	100 days
Untreated Back Bay soil . . . . .	14.0	30 days	100 days
Sterile garden soil . . . . .	3.5	40 days	2 years
Sterile garden soil . . . . .	7.0	30 days	2 years
Sterile garden soil . . . . .	14.0	30 days	2 years

\* Failure of growth due to iron bacteria which grew so abundantly that growth of other plants was prevented.

For the experiments with the other four species of plants mentioned, no tabular results are given. These experiments were set up later and all the checks are still living. The average length of life of the inoculated plants, however, approximates the averages given for *Potamogeton pectinatus*, with the exception of *Vallisneria spiralis*. The absence of a stem above the ground in this plant aids in the prolongation of life, as it is necessary for the fungus to destroy the dozen or more leaves before the plant dies. This plant lived in the inoculated cultures from two to several weeks longer than the species of *Potamogeton*. From the diseased plants of these experiments pure cultures of *Rhizoctonia solani* were re-isolated.

The preceding experiments were run in duplicate with a strain

of *Rhizoctonia* isolated from diseased potatoes. No appreciable differences were observed, however, between the results of these experiments and those in which the aquatic strain of the fungus was used for the inoculations.

### Relation of salt content of water to incidence of the disease

In order to determine the influence of different degrees of salinity of the water upon the *Rhizoctonia* disease, tubers of *Potamogeton pectinatus* were cultured in glass museum jars with boiled Long Island Sound water diluted with distilled water to the following concentrations: no sea water, 3.5, 7.0, 10.5, 14.0, 17.5, 20.0, 25.5, 27.0, 30.5, and 40.0 per cent normal sea water. The concentrations were checked by the titration method described by DENNY (1). No soil was used in this experiment. Three tubers were cultured in each jar, the tubers being weighted down by sterile stones (pl. XII). One of the tubers was inoculated by making a slight cut and inserting sclerotia of the fungus in the wound; a second tuber was inoculated merely by tying sclerotia on to it; the third tuber of each culture was not inoculated. For these inoculations the aquatic strain of *Rhizoctonia* was used. Duplicate experiments were run, using for the inoculations pure cultures of *Rhizoctonia* isolated from diseased potato plants.

*Rhizoctonia* isolated from aquatic plants was found to attack the plants in all salt concentrations employed in the experiment. The disease appeared more virulent, however, in those concentrations between 7 and 20 per cent normal sea water. In none of these cultures did plants live longer than six weeks. All three tubers of each culture became equally infected; injuring the tuber apparently did not aid the infection.

Pl. XII shows four plants two weeks after inoculation with the aquatic strain of *Rhizoctonia*. *G* is a plant cultured in fresh water; *H* in 3.5 per cent normal sea water; *I* in 7 per cent sea water (the concentration of Back Bay); and *J* in 14 per cent sea water. Pl. XIII shows plants of the same cultures, in the same order, four weeks after inoculation. Photographs for these plates were taken through the museum jars and the water solutions in which the plants were cultured.

The strain of the fungus isolated from diseased potatoes attacked the plants in all concentrations used in the experiment. The disease induced by this strain, however, was most destructive to the plants cultured in fresh water and in the concentrations below 10.5 per cent sea water. While plants cultured in the higher concentrations were attacked, death did not occur until long after the plants had fruited. In pl. XIV, *O* represents plants of a 10.5 per cent normal sea water culture; *P* those of a 27 per cent normal sea water culture. Both photographs were taken three weeks after inoculation with *Rhizoctonia solani* isolated from diseased potato. The facts that this strain of the fungus attacks aquatic plants growing in salt solutions of lower concentrations and that the aquatic strain attacks those in the higher concentrations indicate that the two are separate physiological strains of *Rhizoctonia*.

#### Effect of aquatic strain of *Rhizoctonia* upon potatoes

In order to make a more complete comparison between the potato and the aquatic strains of *Rhizoctonia*, it now remained to study the effects of the aquatic strain upon potato plants. For this purpose potatoes were grown in sterile soil under bell jars. A part of the seed pieces were inoculated in the eyes, at the time of planting, with sclerotia of the fungus. Other inoculations were made in the stems of plants just beneath the surface of the soil. Within a short time dark brown lesions, or sunken areas, appeared on the stems of the inoculated plants, and many underground stolons were completely severed. Upon the appearance of the stem lesions the bell jars were removed and the plants were left to grow for several weeks, until tubers had formed. Upon examination, sclerotia of the fungus were discovered on these tubers, many of which were badly scabbed and russeted (fig. 2).

The results obtained in all the preceding experiments with the two strains of the fungus check very closely. For this reason, there is a possibility that the aquatic plants in Back Bay and Currituck Sound have become infected with *Rhizoctonia* from diseased potatoes. While a large district in which potatoes are grown on a commercial scale drains into these waters, and *Rhizoctonia* is known to occur in these potato fields, we do not know that this is the source of



infection. There seems to be just as strong a possibility that the source of the fungus may be found in the great amount of sewage that enters these waters from the city of Norfolk by way of the Albemarle and Chesapeake Canal.

### Seasonal developments of the disease

Evidences of *Rhizoctonia* infection are usually observed each year during the first half of June on the *Potamogeton pectinatus* plants growing in the northern part of Currituck Sound and in Back Bay.



FIG. 2.—Effect of aquatic strain of *Rhizoctonia* upon potatoes

During this month these plants are rather well covered by gelatinous remains of old hydroid colonies which infest the plants a little earlier in the season. At this time of the year the *Rhizoctonia* infections are somewhat limited to dark brown lesions on lower portions of the plant stems, but during the warmer months of August and September the effects of the disease become quite marked in the entire appearance of the plant. We have been able to isolate pure cultures of the fungus from these diseased plants in the months of June, July, August, and September. During the months of August and September the salinity of the water is usually highest (fig. 3). Great

areas of the plants, which up to this time of the season have appeared green, become brown and die within a few days. While the increased salinity of the water may lower the resistance of the plants to the disease, it can hardly be said to kill them, for we have been able to grow the same plants in much saltier water than that found in Back Bay. Furthermore, the dead areas of plants are usually very

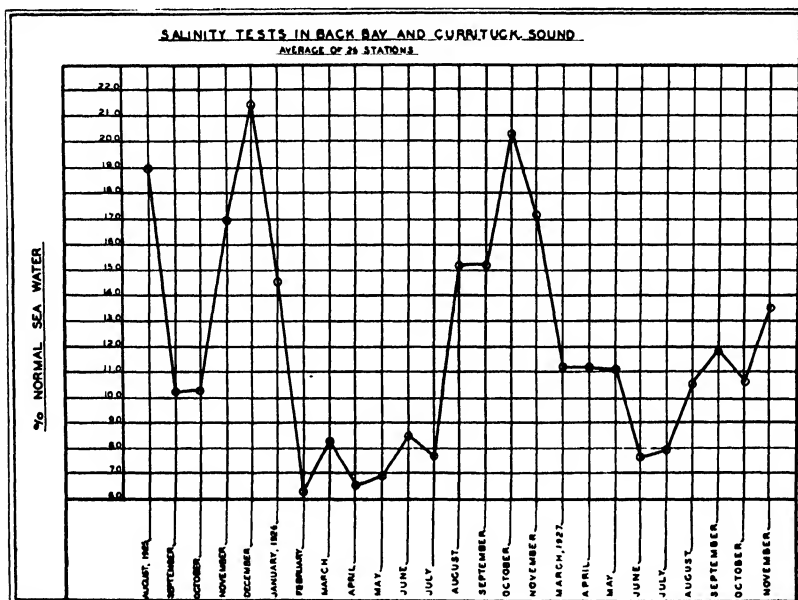


FIG. 3.—Graph showing results of monthly salt analyses of waters of Back Bay and Currituck Sound; readings in percentage of normal sea water, representing averages for 26 widely scattered stations in the region.

irregular, and injuries are restricted to definite portions of the plant itself.

The depth of the water does not effect the development of the disease. *Rhizoctonia* has been found to attack the plants growing in all depths found in Back Bay and Currituck Sound. The depth of these waters, however, does not usually exceed ten feet. In this region there is considerable wind and wave action, and diseased plants, which have lost their anchorage, are easily washed from one area to another. Plants more or less isolated are easily broken off

after infection at the surface of the soil, and are washed ashore while still green; but where the growth is denser the plants remain attached and sink to the bottom after complete decay. In the greenhouse inoculation experiments the plants probably remained attached and alive much longer than they would have done in nature, because wave and wind action was not encountered.

### **Susceptibility of different plants to the disease**

While *Rhizoctonia* has been found to attack most of the species of duck-food plants in Back Bay and Currituck Sound, *Potamogeton pectinatus*, which is the dominant plant of the region, and, incidentally, the most valuable duck-food known, is the most susceptible to the disease. This species is the first to make a growth in the spring, and usually reaches the surface of the water, even in the greater depths, before growth begins in other species of duck-food plants mentioned. Isolated areas of *Vallisneria* and *Naias* do not readily become infected with the disease, but infection does occur when these plants are associated with *Potamogeton*. Next to *P. pectinatus*, the plant most susceptible to the disease is *Ruppia maritima*. In this locality the *Ruppia* zone, while in shallower water, usually overlaps the *Potamogeton* zone. Because of its ease in vegetative propagation, however, *Ruppia* usually survives where the *Potamogeton* dies. Plants of all species studied seem less susceptible to the disease when growing, somewhat sheltered from the winds, in sandy soil.

### **Mycelial and sclerotial characteristics of fungus**

A microscopic examination, as well as a cultural isolation, of the infected portions of the plants reveals hyphae of *Rhizoctonia*. Under natural conditions mycelial threads are seldom so abundant as to be discernible by the naked eye, but are quite visible in aquaria cultures (pls. XII and XIII). The older external hyphae (fig. 4) are somewhat colored, usually a uniform yellowish brown, with branches arising at approximate right angles to the main hypha; the internal hyphae are usually smaller, practically colorless, and the branching is more or less dichotomous. The younger hyphal branches (fig. 5) are practically colorless and contain large globules. Microchemical tests indicate that these globules contain glycogen. The hyphal

branches are almost invariably constricted, and contain a septum near the point of union with the main hypha (figs. 4, 5). The size of the mycelial cells, taken from 100 measurements, is  $50-225 \times 5.50-12 \mu$ . DUGGAR (2) gives  $100-200 \times 8-12 \mu$  for the size of the hyphal cells of *Rhizoctonia solani*. PELTIER (4) found the size of the hyphal cells of the different strains of the fungus was  $65.24-5.01-6.57 \mu$ ,

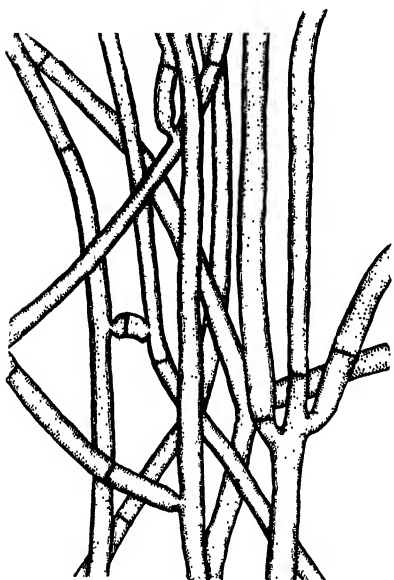


FIG. 4

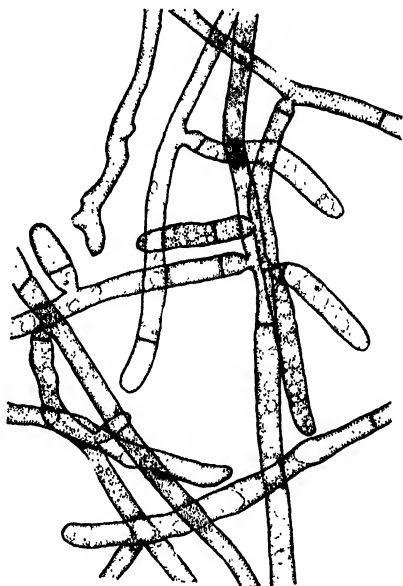


FIG. 5

FIGS. 4, 5.—Fig. 4, *Rhizoctonia solani*, camera lucida drawing of portion of mycelium growing on aquatic plant; fig. 5, *Rhizoctonia solani*, camera lucida drawing of young hyphal branches of mycelium growing on aquatic plant.

but gave for the length of the cells of the strain parasitic upon *Dianthus*  $50-215 \mu$ . PALO (3) states that the measurements of the hyphal cells of the strain parasitic upon rice are  $24-248 \times 4-12.8 \mu$ . The majority of our measurements for the aquatic strain fall within the range given by DUGGAR.

Hyphae of the infecting mycelia readily penetrate the tissues of the aquatic plants. These penetrating hyphae appear greatly swollen at points of entry or exit from the cells of the host tissue, but a very constricted portion of the hyphae penetrates the cell walls of the host plant.

The exact conditions under which sclerotia may occur on the plants in their native habitat have not been determined. Sclerotia have been observed on the dead plants which float ashore in the late summer. In pl. XIV, *Q* is a photomicrograph of a small stem of an aquatic plant containing sclerotia of the fungus. This plant was fixed in chrom-acetic fixative and stained *in toto* with eosin and

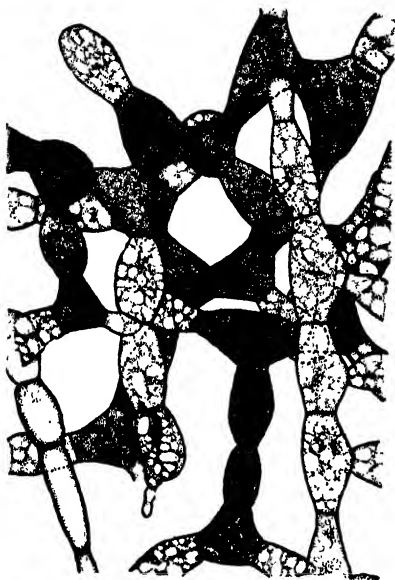


FIG. 6.—*Rhizoctonia solani*; sclerotial cells found on aquatic plant; camera lucida drawing made from stained specimen.

Ehrlich's haematoxylin. Sclerotia first appear on the plants which have washed ashore as light spots bordered by a dark brownish ring of discolored host tissue. These light spots later become dark specks. Some of the sclerotial cells are irregular, flattened, dark, and more or less granular in structure; others, especially of the loose hyphae covering the sclerotia, are barrel-shaped, less colored, and are vacuolate (fig. 6). It is assumed that the sclerotia overwinter in the muck and débris at the bottom of the water. PALO, in reporting the occurrence of *Rhizoctonia solani* upon Philippine rice, states that the viability of the sclerotia is destroyed

after its submergence in water for a period of two to three months. We have succeeded in obtaining infection without re-inoculation in aquaria two years after the soil in these vessels had been inoculated with sclerotia of the aquatic strain of *Rhizoctonia*. This may be due to a prolonged mycelial growth of the fungus, however, as we were unable to determine that sclerotia had formed in the aquaria.

### Summary

1. Great areas of aquatic duck-food plants have been destroyed in Back Bay, Virginia, and Currituck Sound, North Carolina. Di-

rectly or indirectly, this has caused a large economic loss to a large population. A fungus disease has been found the factor most responsible for this loss.

2. Pure cultures of a strain of *Rhizoctonia* have been repeatedly isolated from diseased aquatic duck-food plants collected from the infected waters. A disease, identical with that produced by a strain of *R. solani*, has been produced in greenhouse aquaria plants by inoculations with this aquatic fungus. Pure cultures of the fungus have again been re-isolated from these greenhouse plants. Moreover, a disease has been produced on potato plants by inoculating the plants with sclerotia from culture of this aquatic fungus.

3. This aquatic strain of *Rhizoctonia* has been found to attack five species of aquatic duck-food plants: *Potamogeton pectinatus*, *P. perfoliatus*, *Ruppia maritima*, *Vallisneria spiralis*, and *Najas flexilis*. Attacks have occurred on plants growing in various types of soils, and in waters containing a wide range of salt content. Muck soils and a salinity of 7–20 per cent normal sea water, however, are most favorable for the growth and activity of the fungus. These suitable conditions of soil and salinity now exist in Back Bay and Currituck Sound.

4. Morphological and cultural characteristics indicate that this fungus is a physiological strain of *Rhizoctonia solani*, and it is so considered by the writers.

5. To our knowledge, this is the first case ever reported of the occurrence of *Rhizoctonia solani* on aquatic plants.

6. A strain of *Rhizoctonia solani* isolated from diseased potatoes has been found to attack aquatic plants growing in various types of soils and salt solutions. This disease was identical with that produced by the aquatic strain of the fungus, but occurred in lower salt concentrations.

7. Under favorable conditions *Rhizoctonia solani* is an aggressive parasite upon those species of aquatic plants studied in this investigation, and the disease induced by it has caused much damage to these plants growing in the infected waters.

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## EXPLANATION OF PLATES XI-XIV

## PLATE XI

Three weeks' old cultures of *Rhizoctonia solani*: *A* and *B*, cultures of fungus isolated in 1926 from diseased aquatic plants collected from Back Bay, on potato agar and on green string bean agar respectively; *C* and *D*, cultures on potato and green string bean agar respectively, of aquatic strain of *Rhizoctonia* re-isolated in 1927 from greenhouse aquatic plants used in inoculation experiments; *E* and *F*, cultures of *R. solani* isolated from diseased potato plants, on potato and on green string bean agar respectively.

## PLATE XII

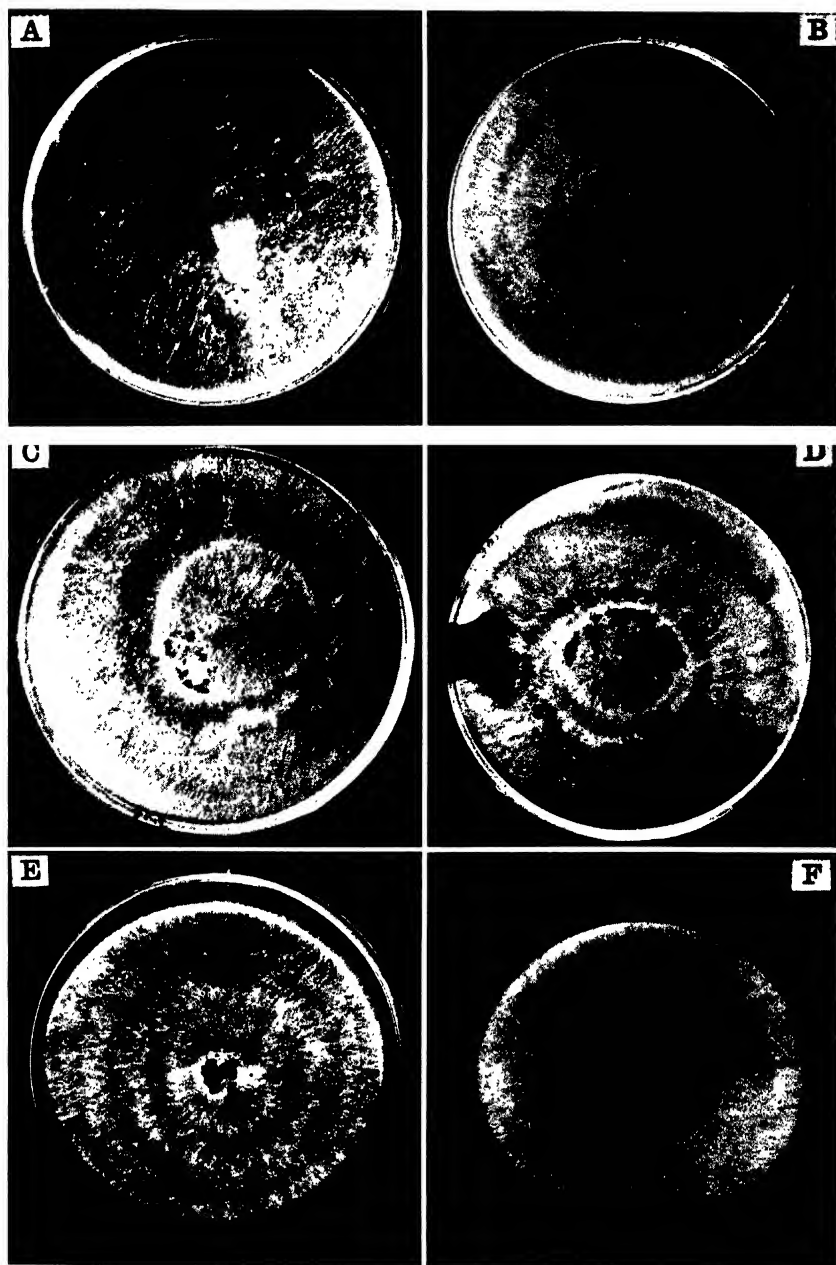
*Potamogeton pectinatus* plants two weeks after inoculation with sclerotia of aquatic strain of *Rhizoctonia*; *G*, plant cultured in fresh water; *H*, in 3.5 per cent normal sea water; *I*, in 7 per cent.

## PLATE XIII

Same as pl. XII four weeks after inoculation; these are plants from the same cultures and arranged in same order as in pl. XII.

## PLATE XIV

*Potamogeton pectinatus* plants (*O*) growing in 10.5 per cent normal sea water three weeks after inoculation with sclerotia of *Rhizoctonia* isolated from diseased potato plants; *P*, same as preceding except plants are growing in 27 per cent sea water; *Q*, photomicrograph of sclerotia of aquatic strain of *Rhizoctonia* on small stem of *P. pectinatus*; plant found washed ashore in Back Bay, Virginia; specimen fixed in chrom-acetic fixative and stained with eosin and Ehrlich's haematoxylin.



BOURN & JENKINS on RHIZOCTONIA







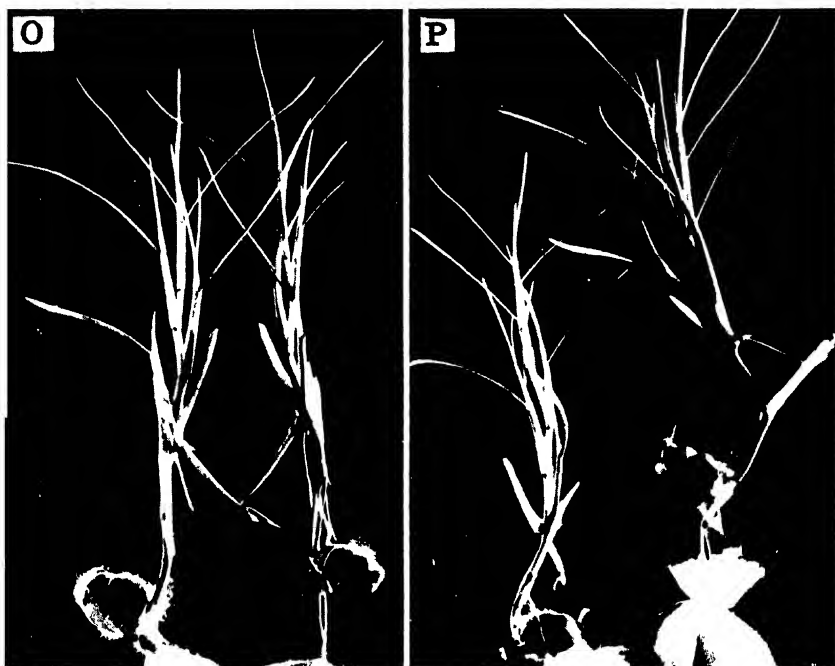
BOURN & JENKINS on RHIZOCTONIA





BOURN & JENKINS on RHIZOCTONIA





Q



BOURN & JENKINS on RHIZOCTONIA



# ANATOMICAL STUDY OF PLANTS GROWN UNDER GLASSES TRANSMITTING LIGHT OF VARIOUS RANGES OF WAVE LENGTHS<sup>1</sup>

NORMA E. PFEIFFER

(WITH PLATES XV-XVIII AND ONE FIGURE)

A series of plants was grown in glass houses at Boyce Thompson Institute for Plant Research in the summer of 1927, for physiological experiments. A few of the plants were available for anatomical study, the results of which are here recorded. The houses in which the plants were grown have been previously described and figured by POPP.<sup>2</sup> Briefly, each house is 9×11 feet, with roof sloping to the south, with a difference of 2 feet in height at front and back of benches. The ventilation system is of the positive pressure type, electrically driven, with separate air intakes in each house and separate roof ventilator outlets. This results in temperatures which are approximately similar throughout the line of houses, although not identical with that existing out-of-doors in full sunlight.

## Transmission of glasses used in houses

The glasses used in the houses in this experiment transmit light in the visible spectrum and in the ultra-violet in various ranges of wave length. The designations used for these houses, the trade name of the glasses,<sup>3</sup> and the ranges of each within these two spectral regions are as follows:

	Millimicrons
Visible-spectrum house.....Noviol O.....	720-390
Full-spectrum house.....Corex.....	720-290
Blue house.....G403ED.....	585-335
Minus-violet house.....Noviol C.....	720-471
Red house.....G 34.....	720-526

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

<sup>2</sup> POPP, HENRY W., A physiological study of the effect of light of various ranges of wave length on the growth of plants. Amer. Jour. Bot. 13:706-736. 1926.

<sup>3</sup> Corning glasses.



The spectrum of sunlight in the same regions is from 720 to 400 millimicrons in the visible spectrum, and from 400 to 290 millimicrons in the ultra-violet. The light in the full-spectrum house most nearly approaches this in quality of rays, transmitting the ultra-violet rays to the extent of about 80 per cent of their value in full sunlight. The blue house is limited in transmission in the visible spectrum to some yellow, green, and blue, with less rays in the ultra-violet than window glass transmits. The minus-violet house allows the passage of the red, yellow, green, and part of the blue of the visible spectrum. In the red house, only red, yellow, and part of the green of the visible spectrum are transmitted. The last two houses mentioned lack all ultra-violet rays, the visible-spectrum house most of them, and the blue house glass transmits less than window glass. Thus all four fail to transmit the rays valuable to higher animals because of their antirachitic effects. The blue and red house glasses transmit in common only some yellow and some green rays.

In addition to these five glass houses, two other locations served for comparison. One was an adjacent platform, commonly termed "outdoors," where plants received full insolation. Another was a "shade" house, a light frame structure covered with a double layer of gauze. This stood between the spectral glass houses and the "outdoors" plants, also having a southern exposure. These two situations did not have the similarity of temperatures that obtained in the glass houses.

### Intensities

The measurements of intensities are those made by J. M. ARTHUR and H. L. SHIRLEY, using the Macbeth illuminometer and a pyrheliometer. The latter records radiant energy in terms of heat; the former is more specifically a measure for illumination. It is not entirely satisfactory, however, in that the eye, by means of which intensities are gauged, is not ideal in its ability to distinguish degrees of difference equally in various regions of the spectrum. Moreover, the color effect within the glass houses makes accurate readings even more difficult. Neither instrument records light in terms of the plant and physiological effects. Table I represents the averages of readings made at various times, based on the two methods. The light outdoors in each case was considered as 100 per cent intensity,

whether in terms of foot candles (Macbeth illuminometer) or millivolts (pyrheliometer).

### Anatomical studies

From the range of plants available in the different sets of conditions, several were selected for a study of the anatomical effects produced by the light factor. So far as possible, plants had been grown from seed, although when necessary small plants, as 1-inch salvias, represented the stage at the beginning of the experiment. In all cases 2-gallon jars with forest soil were used for the plants. The beginning date of the experiment was June 13, 1927.

TABLE I  
INTENSITIES IN THE VARIOUS SITUATIONS

SITUATION	MACBETH ILLUMINOMETER Per cent	PYRHELIOMETER Per cent
Outdoors.....	100	100
Visible-spectrum house.....	59.8	52.7
Full-spectrum house.....	55.0	50.8
Blue house.....	8.6	8.0
Minus-violet house.....	50.7	33.2
Red house.....	31.3	30.2
Shade house.....	16.2	23.2

STEMS.—When the plants were six weeks old, the stems of a number were examined, especially *Mirabilis jalapa*, *Brassica rapa* (Henderson's Purple Top White Globe), *Helianthus cucumerifolius* (type of Henderson), and *Glycine soja* var. *biloxi*. The first two proved least satisfactory for accurate comparison of degree of development of vascular tissues, because of the scattered arrangement of unequal bundles in four-o'clock, and because of the short stem in turnip which makes it difficult to get comparable preparations. Yet it was possible to see in these the same general effects that were more readily demonstrable in the typically dicotyledonous stems of the sunflower and soy bean. Later collections, made when the plants were approximately fourteen weeks old, again included these two forms.

Free-hand sections were made of the stem at the base and just below the middle node. These show similar trends, as may be seen in the illustrations from photomicrographs, taken at the fourteen-week

stage (pls. XV-XVIII). Study was made of two stems, of which one set was photographed. In comparing results, the full-spectrum house is considered the control, since it has the spectrum of sunlight and the temperature is similar to that of the other four houses. The different temperature outdoors and in the shade house bring in another factor which would eliminate the use of these as checks.

In soy bean (pls. XV and XVI) the stem in the full-spectrum house compares very favorably with that outdoors, and is noticeably stronger in development of woody tissues than in any other house. The plants in the shade house and minus-violet house are much alike, both a little less strong than those of the visible-spectrum house. Undoubtedly the plants in the blue and red houses represent the weakest development. That the least differentiation occurs in the blue house may be due as much to the very low intensity as to the special rays which reach the plant.

In the case of the sunflower (pls. XVII and XVIII) the full-spectrum house plant is not so strong in development of differentiated tissues as either the outdoors plant or that of the visible-spectrum house. There is little difference in intensity in the two houses, but that little is in favor of the visible-spectrum location. Whether this could be of more importance to a "sun" form like *Helianthus* than to other types, it is impossible to say. The plants in the minus-violet house also had well developed xylem, although here intensity is less high. It would have been interesting to compare the shade form in this case, but unfortunately all plants were used before the final anatomical study. The earlier stage showed weaker vascular tissues under these conditions than the minus-violet plants. In all cases the plants in the blue and red houses were least differentiated. In the upper region the former showed no secondary wood, although at a corresponding level the latter had a fair amount. With the low intensity in the blue room, it is very possible that the material for elaborating differentiated tissues is lacking. It is conceivable that the available food may be the limiting factor, and that the quality of light is not directly responsible for such weak development. Certainly the appearance of the plant, a sort of miniature of those in the full-spectrum house, is more nearly normal than that of plants in visible-spectrum, minus-spectrum, or red houses.

Aside from the actual amount of differentiated tissues produced, the dimensions of the stem prove of some interest. Considering the diameter of the base of the stem of the full-spectrum house as 100 per cent, we get the results shown in table II. Here the sunflower and four-o'clock appear to respond in similar fashion, with the exception of sunflower in the outdoors situation. Compared with these two, the soy bean makes a better showing, so far as diameter is concerned, in both the red house and in shade. This is not necessarily due to development of differentiated tissue, but chiefly to pith.

TABLE II  
DIAMETER OF BASE OF STEMS (SIX WEEKS OLD) AS COMPARED WITH  
FULL-SPECTRUM HOUSE

HOUSE	FOUR-O'CLOCK Per cent	SUNFLOWER Per cent	SOY BEAN Per cent
Outdoors.....	95	150	97
Visible-spectrum.....	82	81	70
Full-spectrum.....	100	100	100
Blue.....	56	50	60
Minus-violet.....	78	75	70
Red.....	43	49	67
Shade.....	66	74	83

It is well to associate height with diameter. In table III the same plants considered in the previous table are listed on this basis, again with the full-spectrum plant as 100 per cent. Here also sunflower and four-o'clock appear more nearly parallel, whereas soy bean reacts quite differently. It endures low intensities better than the other two forms, as shown by diameter and height. On the other hand, where abnormal form is brought about by lack of the blue and ultra-violet rays, soy bean is even more affected (as shown by twining) than the other two genera.

Table IV records a comparison of heights from the more extensive physiological data collected by J. M. ARTHUR. The previous table is based on two samples, while this is an average of a greater number (2 to 7 more) of plants. In table V there is the same general trend, although minor differences appear in the figures.

LEAVES.—In form the leaves in the minus-violet and red houses appear puffed and warped, although they are ordinarily of very good

size. In the blue-house plants, on the other hand, the greatest reduction in leaf expanse runs parallel to the small size of the whole organism. The leaves in the other houses, which are intermediate between these two extremes, fall into this order, on the basis of expanse: second largest in visible and full-spectrum houses, next in outdoors and shade houses.

TABLE III  
HEIGHT OF STEMS (SIX WEEKS OLD) AS COMPARED WITH  
FULL-SPECTRUM HOUSE; SAME STEMS AS TABLE I

HOUSE	FOUR-O'CLOCK Per cent	SUNFLOWER Per cent	SOY BEAN Per cent
Outdoors.....	85	85	57
Visible-spectrum.....	104	100	144
Full-spectrum.....	100	100	100
Blue.....	57	26	91
Minus-violet.....	100	110	181
Red.....	110	49	185
Shade.....	54	66	115

TABLE IV  
AVERAGE HEIGHT OF STEMS (SIX WEEKS OLD) AS COMPARED WITH  
FULL-SPECTRUM HOUSE

HOUSE	FOUR-O'CLOCK Per cent	SUNFLOWER Per cent	SOY BEAN Per cent
Outdoors.....	103	95	60
Visible-spectrum.....	115	98	149
Full-spectrum.....	100	100	100
Blue.....	53	25	82
Minus-violet.....	103	97	141
Red.....	120	46	168
Shade.....	83	65	100

In considering the thickness of leaves, the extremes fall in the outdoors plants, where the leaves are thickest, and in the red house, where they are least thick. In two of the three forms measured (soy bean and four-o'clock), those growing in the full-spectrum house rank highest among the houses, that is, most nearly approaching outdoors plants; while the visible-spectrum plants made a close second. In the case of sunflower, the relation in these two houses is

just reversed. The minus-violet and shade leaves tally with each other very well, and the blue-house leaves, with their small size, prove but little different from these. The order here is (from the thickest to least thick): full-spectrum, visible-spectrum, blue, minus-violet and shade, and red-houses. The shade and minus-violet sunflowers are aberrant.

Here it is obvious that quality of light is effective in reducing the thickness of the red-house leaves, since the intensity (table I) is intermediate between that of the blue house and the minus-violet house, both of which have thicker leaves.

TABLE V  
THICKNESS OF LEAF

HOUSE	FOUR-O'CLOCK Per cent	SUNFLOWER Per cent	SOY BEAN Per cent
Outdoors.....	110	154	133
Visible-spectrum.....	84	107	80
Full-spectrum.....	100	100	100
Blue.....	68	79	77
Minus-violet.....	66	100	66
Red.....	48	79	63
Shade.....	66	102	66

In studying the differentiation of the leaves, one finds that in sunflower and soy bean two layers of palisade cells ordinarily develop in outdoors conditions (fig. 1). In this feature, the full-spectrum leaves resemble those outdoors very definitely; while in the visible-spectrum house, lacking the ultra-violet rays, the second layer is weaker in soy bean. In all the other houses there is reduction to one layer, and in shade, with ultra-violet rays available, although in low intensity, we again find a vague second layer produced.

In four-o'clock (fig. 1) the long compact palisade layer, seen in outdoors leaves and in full and visible-spectrum leaves, does not develop in the other houses. In the shade house it is of intermediate length, while the cells in the minus-violet house are even shorter; in the blue house they are very open; and in the red, very short. In all cases leaves in the blue and red houses show a markedly poorer development than those in full or visible-spectrum houses. The weakest differentiation occurs in the red house.

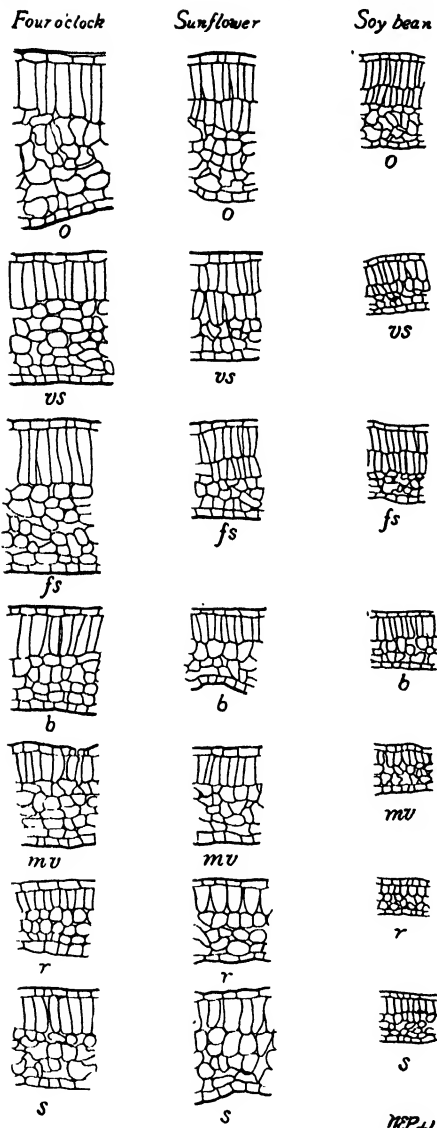


FIG. 1.—Sections of leaves of four-o'clock, sunflower, and soy bean from plants grown in different houses; houses are indicated by the following letters: *o*, outdoors; *vs*, visible-spectrum house; *fs*, full-spectrum house; *b*, blue house; *mv*, minus-violet house; *r*, red house; *s*, shade.

**ROOTS.**—It seems worth while to consider roots, but so far the material available renders it difficult to make any statement based on measurements, such as were made of the other organs. One may make an estimate on the basis of length, thickness, and development of secondary roots. Using this method, it was found that the outdoors plant takes precedence over any of the glass-house plants. Of the latter, the full-spectrum plant ranks first, the visible-spectrum next, the minus-violet plant (to which shade plants are similar) third, with the plants in the blue and red houses decidedly poor and feeble in comparison.

### Discussion

It is difficult to make valid comparisons of plants in different situations where more than one factor varies. Hence the first two houses, which have approximately equal intensities, are the best ones on which to base conclusions as to the effect of quality of light. It seems evident that the plant in the full-spectrum house makes a better growth, so

far as differentiated tissues (as shown in vascular tissues in stem, diameter of stem, and thickness of leaf) are concerned; and it produced the usual form of plant, with the tissues commonly found in full insolation. The plant in the visible-spectrum house is weaker in vascular development, more spindly as to stem, with greater height, less thick as to leaf, and with poorer root development. The difference in light, that is, lack of most of the ultra-violet rays, results in this less stocky, less sturdy, more watery plant.

The same effect on form is noticeable in the two houses which emphasize the red end of the spectrum. Here, however, different

TABLE VI

SUMMARY: DEGREE OF DEVELOPMENT LISTED IN ORDER WITH THE HIGHEST FIRST

STEM			LEAF		ROOT
Vascular development	Diameter	Height	Thickness	Differentiation	Development
Outdoors.....	Full-spectrum	Visible-spectrum	Outdoors	Outdoors	Outdoors
Full-spectrum...	Outdoors	Full-spectrum	Full-spectrum	Full-spectrum	Full-spectrum
Visible-spectrum...	Visible-spectrum	Minus-violet	Visible-spectrum	Visible-spectrum	Visible-spectrum
Minus-violet....	Minus-violet	Outdoors	Blue	Shade	Minus-violet
Shade.....	Shade	Blue	Minus-violet*	Blue	Shade
Blue or red....	Blue or red	Red and shade vary in position	Shade*	Minus-violet	Blue
Red or blue....	Red or blue		Red	Red	Red

\* Exception in sunflower.

intensities, that is, lower ones, enter in as a factor, and may be partly responsible for the very weak stems, for the poor differentiation and thinness of leaf, and for the slight development of root.

It is unfortunate that in the blue house it has not been possible to obtain an intensity more nearly comparable with that of the full-spectrum house. At present it is impossible to dissociate the effects of quality and intensity of light in this house. In form the plants appear like miniatures of full-spectrum plants. Microscopic examination, however, shows very much weaker differentiation and development of tissue in stem and leaf. Whether the amount of food is the real limiting factor, that is, an indirect effect of light, or whether the light itself directly determines this, is not known.

### Summary

Some sort of diagram, which would bring all these organs and their development into one view, seems a useful sort of summary.



Table VI serves this end. Usually outdoors plants take the first place, with the full-spectrum plants usually second, and visible-spectrum plants third. Except in height, which is the least tractable of the figures to handle, minus-violet-house and shade plants usually fall close together, and the blue- and red-house plants are generally at the end of the series, showing weaker development and differentiation. In the full-spectrum and visible-spectrum houses quality of light determines the difference, since the intensities are similar. In the remaining houses, both quality and intensity may be effective in bringing about the changes.

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#### EXPLANATION OF PLATES XV-XVIII

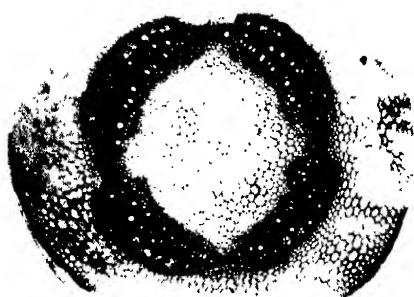
All photomicrographs of free-hand sections of stems were made with the use of the Leitz Makam micro-camera. In all plates the first column, *B*, shows the base of stem; the second, *M*, shows the middle of stem, half-way between base and apex. The smaller letters indicate locations in which the plants were grown: *o*, outdoors; *vs*, visible-spectrum house; *fs*, full-spectrum house; *b*, blue house; *mv*, minus-violet house; *r*, red house; *s*, shade. Plants were 14 weeks old.

##### PLATES XV, XVI

*Glycine soja* var. *biloxi*.

##### PLATES XVII, XVIII

*Helianthus cucumerifolius*, type of Henderson.



*U S*



*B*

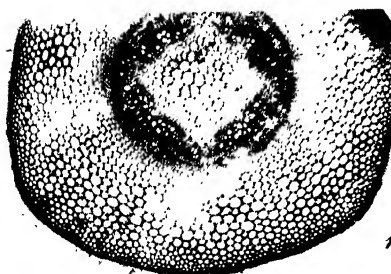
*f s*

*M*

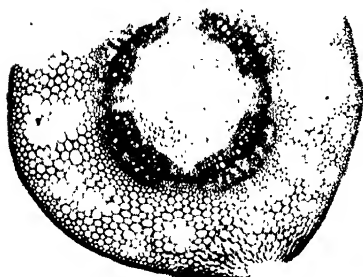




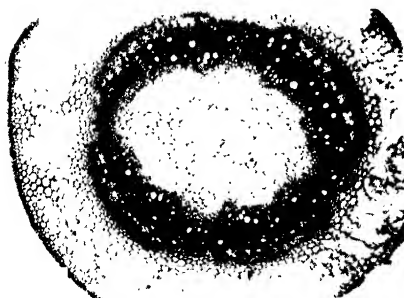
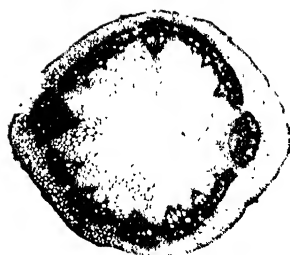
*b*



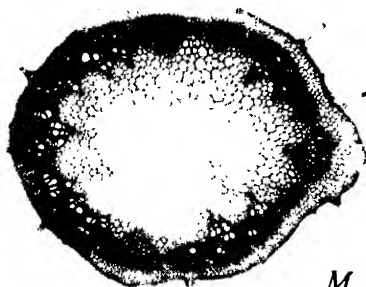
*m v*



*r*



*s*

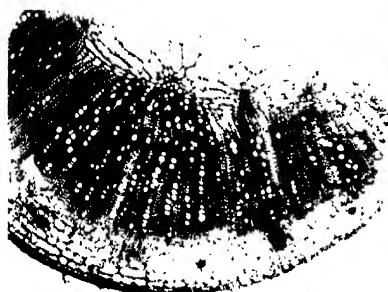


*M*

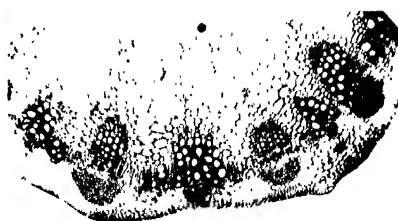
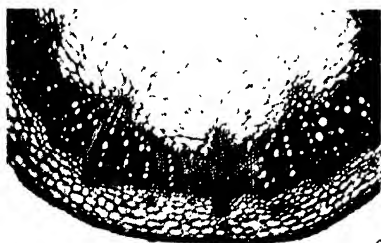




*o*



*v s*



*f s*

*B*

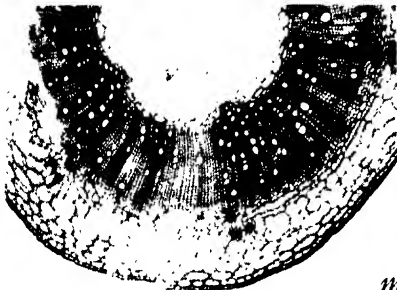
*M*

PFEIFFER on PLANTS AND LIGHT

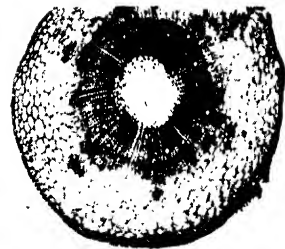
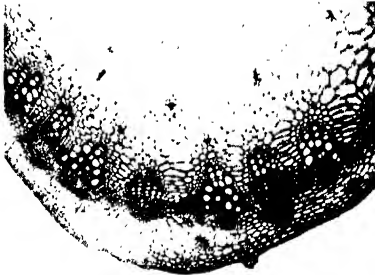




*b*



*m v*



*r*



*B*

*M*





# MALE GAMETOPHYTE OF MICROCYCAS CALOCOMA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 381

DOROTHY G. DOWNIE

(WITH PLATES XIX-XXI AND ONE FIGURE)

## Introduction

In 1907 CALDWELL and BAKER (2) published a taxonomic account of *Microcycas calocoma*, and a few months later the first author discussed its distribution in the field and described it morphologically (1). CALDWELL made no attempt to trace the development of either male or female gametophyte, as his material was inadequate; but he made the remarkable discovery of the occurrence of large numbers of sperms in the pollen tube, and correlated this with the increased development of archegonia in the female gametophyte. Since these publications, material has accumulated in this laboratory from collections made in the western Sierras of Cuba by CALDWELL in 1909, 1910, and 1911, covering a period from November 21 to April 24; by CHAMBERLAIN in September 1922; and by the writer in August and September, 1927. In 1924 MISS REYNOLDS (9) published an article on the development of the female gametophyte of *Microcycas calocoma*, and in 1926 Professor CHAMBERLAIN suggested to the writer that the development of the male gametophyte offered a fruitful field for further study.

The occurrence of many sperms in an antheridium is a feature of bryophytes and pteridophytes, in contrast with the two-sperm or male cell condition in the much reduced male gametophyte of the spermatophytes. Thus the development of the pollen grain in *Microcycas calocoma* might be expected to throw some light on the transition from the antheridium of the pteridophytes to the pollen tube of the seed plants. This study was undertaken, therefore, with a view to tracing the development of the male gametophyte and comparing it with that of other Cycadaceae; and also with the antheridial development of the leptosporangiate ferns. Progress in the bryophytic and pteridophytic antheridia is toward a gradual reduc-

tion in the primary spermatogenous cells, and in the output of spores. In the pteridophytes this is correlated with a reduction in the prothallial tissue. It was important, therefore, to demonstrate the number of primary spermatogenous cells, the generation of the sperm mother cell with reference to this, and the extent of the prothallial tissue. A brief examination of the pollen tubes indicated favorable material for the study of the blepharoplast, and it was hoped that in the division of the spermatogenous tissue the presence or absence of centrosomes could be established.

**POLLINATION.**—Field studies in Cuba show that pollination takes place during the last week of August and the first two weeks of September. By August 28 many male cones were shedding pollen and the female cones showed open cone scales. Examination of the ovules at this time, however, showed no development of the pollen chamber. By the second week in September the pollen chamber had appeared as a narrow vertical slit at the tip of the nucellus, and contained many pollen grains. As in other cycads, the pollen is shed in the three-celled condition, one prothallial, one generative, and one tube cell.

**POLLEN TUBE.**—The number of germinating pollen grains in one ovule is decidedly variable. While in general there are only one or two, as many as twenty-two have been counted in a pollen chamber. The exine of the pollen grain is almost uniform, tapering slightly to the point of exit of the intine, and it is very faintly etched with short parallel markings. The intine which grows out to form the pollen tube is thin at the grain end, but thickens and widens considerably just beyond the exine. It remains an almost uniform thickness for the rest of its length, and is irregularly lamellate. The pollen tube in its course penetrates the cells of the nucellus and absorbs their contents. This penetration is effected by a mucilaginous substance which arises from a transformation of the tube wall (figs. 9, 10). Occasionally the mucilage is found within the tube between the cytoplasm and the intine, suggesting that the substance may be, in part, secreted by the cytoplasm of the tube (fig. 11). The mucilage lies close to the cell wall of the adjacent nucellar cell, and appears to dissolve the cellulose of the wall, so that it becomes perforated and reacts differently to stain (fig. 8). Later notches appear (fig. 9),

which increase in size until the wall is penetrated. The solution is probably effected by enzyme action, but so far no tests have been made to determine the nature of the enzyme or the mucilage. The tube may attack the whole length of the wall at once, but more often its efforts are concentrated on a limited area, and once this is weakened sufficiently, the pressure of the tube bursts the wall (fig. 12). A protuberance then grows into the cell, gradually invading and finally filling it completely. Most of the cell contents are absorbed, but along the wall of the cell there collects a residue of undigested material, which takes up the iron-haematoxylin stain very deeply. This residue apparently acts as a barrier to the absorbing action of the mucilaginous intine, as the progress of the tube is often diverted. An area just behind the advancing point then secretes mucilage, and another cell is attacked in the same manner. The progress of the tube is thus slightly irregular, and this irregularity is enhanced or decreased according to the course of the tube. Generally the tube travels under the epidermis, one, two, or three layers deep in the tissue. The cells of the nucellus are elongate in this region, and a tube following the long axes of the cells meets comparatively little resistance. The result is a tube of fairly uniform diameter, with few or no protuberances or branches and only one effective point of advance (fig. 1). Occasionally a tube takes a more direct course toward the female gametophyte, that is, through the center of the nucellus. Here it meets isodiametric cells, which present a greater number of intervening cross walls per unit length traversed, and the tube is thus evolved into a wide irregular structure with several effective advancing points.

The tube nucleus occupies a position just behind the haustorial end of the tube, about 0.1 mm. distant in older tubes. It has a large nucleolus, surrounded at first by a dense chromatin network, which later becomes very loose and reacts rather faintly to the iron-haematoxylin stain. The nucleus finally becomes irregular in shape and often distinctly lobed. The cytoplasm of the tube is highly vacuolate. The vacuoles are large, and in the interstices collections of small granules, which stain deeply, often occur. In the youngest material starch is prevalent throughout the tube (fig. 3). Later it becomes scarce in the region of the body cells (figs. 5-8), but re-

mains abundant just behind the actively invading zone of the tube (fig. 12). When tube invasion of the nucellus ceases, and the tube nucleus passes back to the body cell region, starch accumulates at the grain end again, occurring as large spherical granules densely aggregated in the tube and in the prothallial and spermatogenous cells (fig. 18). At this time the free end of the tube is much swollen and the intine is stretched and thin, while the body cells are actively dividing. On the division of the body cells and the formation of the sperms the tube ruptures and the sperms escape into the pollen chamber.

**PROTHALLIAL CELL.**—At pollination, and during the early development of the tube, the prothallial cell has a dense cytoplasm and a well defined nucleus (figs. 2-4). Later the cytoplasm becomes diffuse, owing to the formation of large vacuoles; and the nucleus, although large, stains deeply with iron-haematoxylin, almost suggesting disorganization (figs. 7, 8). A tube stained for the study of the body cells is too deeply stained for critical examination of the prothallial and stalk cells. The nucleus of the prothallial cell sometimes shows a slight activity, entering into a late prophase stage (figs. 6, 13). This activity is manifest between successive divisions of the spermatogenous cell, but no division takes place.

By December the prothallial cell has increased considerably in size and has grown well into the stalk cell (figs. 14-17). The cytoplasm has become denser and the nucleus stains normally. What at first was a common dividing wall between stalk and prothallial cells has now split into two distinct walls, which remain in close juxtaposition (figs. 13, 14). While in all other Cycadaceae, where this phenomenon has been described, the prothallial cell grows until it presses against the farthest wall of the stalk cell, in *Microcycas* it does not halt there, but grows right through the wall as a long narrow papilla extending among the body cells (figs. 15-17). This passage of the prothallial protuberance through the stalk is not accompanied by any violent rupture of the latter. The stalk cell remains turgid and appears as a closely investing funnel cell. It would appear that the prothallial cell carries forward with it the basal wall of the stalk cell, until the opposing walls of the latter meet and fuse. Further growth of the prothallial cell then ruptures the fused

wall, without disturbing the cytoplasmic contents of the funnel-shaped stalk cell. The protruding papilla grows until it is almost equal in length to the normal part of the cell. It increases also in width, and finally expands so much that it bulges over the stalk cell. The nucleus always remains in the lower part of the cell, and is surrounded by a highly vacuolated cytoplasm, in which numerous spherical starch grains are imbedded (fig. 18).

The renewed activity of the prothallial cell seems correlated with a period of quiescence in the stalk cell. This situation will be discussed further, therefore, at the close of the description of the stalk cell.

**GENERATIVE CELL.**—At pollination the generative cell resembles the prothallial cell in appearance, and is separated from it by a common wall (figs. 2, 3). Later it increases in size and appears to fit like a cap over the lenticular prothallial cell. After enlarging, it divides to give rise to a body cell and the stalk cell (fig. 4).

**STALK CELL.**—The stalk cell has a characteristic shape, and practically fills the tube at the grain end, but does not extend beyond the narrow zone inclosed by the exine in the September-October material. The cytoplasm of the cell is fairly dense, and contains a few scattered granules which stain deeply. The nucleus is large and slightly excentric in the cell, and the nucleolus is surrounded by a large hyaline area (fig. 6).

The name "stalk cell" was originally given to this cell because it was thought to be the same morphological unit as the stalk cell in the antheridium of the bryophytes and the pteridophytes. This cell is sterile throughout the gymnosperm phylum, with very few exceptions; and this fact, together with its position between the prothallial and the fertile spermatogenous or body cell, gave rise to this conception. Within recent years doubt has been placed on this analogy, but nevertheless the name has been retained in the literature. The writer's findings in *Microcynas calocoma* refute this analogy entirely, and prove, I believe, that the stalk cell is a sterile spermatogenous cell; for in this species the so-called stalk cell is an actively dividing cell, capable of as many as ten or eleven divisions, and cutting off at each division a body cell (figs. 5, 7, 8). This activity takes place during the months of September and October, and follows immedi-

ately the division of the generative cell. I would therefore regard the generative cell as a primary spermatogenous cell, comparable to the cell so named in the lower groups, and the stalk cell as a spermatogenous cell active in *Microcycas calocoma*, but having lost the division potentiality in the other gymnosperms. Such a loss is foreshadowed in *Microcycas calocoma*, where the number of body cells varies from eleven to eight. It should be noticed that the successive divisions of the stalk or spermatogenous cell take place in the same plane, and that a linear arrangement of the body cells in basipetal succession is thus formed.

One interesting exception to this linear arrangement of body cells of increasing age and size was encountered. In this case there appeared two spermatogenous cells in the following order: prothallial cell, spermatogenous cell (1), body cell (1), spermatogenous cell (2), body cell (2), body cell (3), (text fig. 1). Body cell (2) was obviously derived from spermatogenous cell (2), while body cell (3) might have been derived from either spermatogenous cells (1) or (2), although more probably from (2). It would appear that, on the division of the primary spermatogenous or generative cell, both daughter cells had retained the power of division, or that the daughter cell of the second division had retained this potentiality. No other tube has been found showing this variation. It may be that this exception is of historical significance, pointing to a loss in the division potentiality of the daughter cells, the body cells, suggesting at one time an even greater number of spermatogenous cells and a correspondingly larger number of sperms.

The demonstration of centrosomes in the spermatogenous cells of *Marsilia* (10) suggested the possibility of this structure appearing in *Microcycas calocoma* during the division of the "stalk" cell. A careful study of this cell in division and in the resting stage failed to reveal such a body. The rounded end of the spindle might help to confirm the absence of centrosomes.

About the end of October the spermatogenous cell becomes sterile, and it enlarges and grows out of the grain end of the tube. The prothallial cell has also started to grow, and in pushing its way through the covering cell, it displaces the nucleus of the spermatogenous cell to one side. The manner of the subsequent growth of

both these cells has already been discussed. Although enlarging greatly, the "stalk" cell fails to keep pace with the prothallial cell. Both cells at this time are packed full of starch. The further history of these two cells could not be determined, but in all probability, coincident with the bursting of the tube they also burst and discharge their contents.

As an explanation of the occurrence of the body cells in a basipetal series (figs. 6-8), Professor LAND put forward the following suggestions. First, the linear arrangement of the body cells is due to the configuration of the tube, and second, the activity of the spermatogenous cell is dependent on the course of the food stream. He is under the impression that in some way nutrition is inhibited from entering the spermatogenous cell directly, and that therefore it receives its supply by way of the prothallial cell. Always the daughter cell in juxtaposition to the prothallial cell obtains a greater

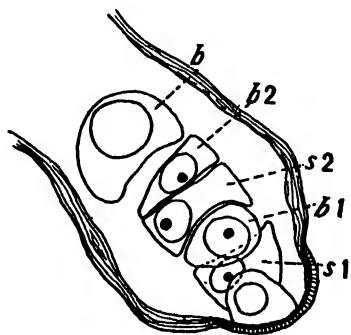


FIG. 1

supply of nourishment, and its growth is therefore more rapid than the other daughter cell (fig. 5). In contrast to the slow growth of the body cells, there is a rapid development of the stalk or spermatogenous cell, so that it is soon stimulated to divide again. The absence of starch at the grain end of the tube in the early stages made it impossible to test out the second part of this theory, but I think the phenomenal development of the prothallial cell, in the later stages of its history, almost establishes this explanation as a fact. For as long as the spermatogenous cell was actively dividing it made great demands on the prothallial cell, but once the former entered into a period of comparative quiescence, the latter, still receiving large supplies, was enabled to grow rapidly. Both obtain unlimited food supply, as shown by their starch content; but the prothallial cell exhibits the more phenomenal increase, easily surpassing its neighbor. This would appear the normal sequence of events if the food stream entered the spermatogenous cell by way of the prothallial cell.



**BODY CELL.**—Owing to the oblique spindle of the spermatogenous cell in division, the body cell is at first wedge-shaped in longitudinal section. The nucleus is large, excentric, occupying the broad end of the wedge, and extending the full width of the cell in one direction. The cytoplasm is fairly dense, the vacuoles small, and granules that stain black with iron-haematoxylin are scattered through the cytoplasm or grouped at the interstices between the vacuoles. Starch is absent. Two blepharoplasts soon appear in the cytoplasm of the body cell at opposite poles (figs. 6, 7).

The body cell and its nucleus increase greatly in size, the cell becoming first oblong, and then rounded on the side away from the grain. Finally it becomes completely spherical, and remains so during the rest of its development, only continuing to increase in size. The nucleus and the blepharoplasts enlarge considerably. In the tube a regular series of body cells is present, from the youngest, next the spermatogenous cell, to the oldest at the tube end (figs. 6–8). Only one linear row is found, emphasizing the fact that the spermatogenous cell divides always in one plane. Several months later, just before the formation of the sperm mother cells, this serial arrangement is lost. The tube widens at the free end, and the spherical body cells, alike in size and structure, are scattered or grouped in a mass. Apparently growth in the younger body cells is more rapid than in the older, so that a universal developmental stage is reached just before division. The rapid enlargement of the spherical body cells in the limited confines of the tube thus causes a displacement of the linear row. The intine is stretched, and is seen to inclose a mass of similar cells which divide almost simultaneously to form sperm mother cells.

**BLEPHAROPLAST.**—The cytoplasm of the young body cell, just after it has been cut off from the spermatogenous cell, is uniform. Soon small granules appear which stain black with iron-haematoxylin. They are scattered in the cytoplasm, and appear to have no relationship to the nucleus. Later they collect in groups (fig. 20), and a definite blepharoplast suddenly appears. As first observed, it seems to be composed of a tiny aggregate of these black granules (figs. 19, 21). The small granules fuse into larger ones, and into a uniform mass which becomes conspicuous in the cell (fig. 22). The

aggregate of small granules appears to be an attractive center, as there soon arise granular stream lines flowing into it on all sides. It appeared that the blepharoplast was formed by the flowing together of these small granules which arise in the cytoplasm. There may be a few small aggregates, but one takes precedence and acts as a center for the stream lines of granular material. One blepharoplast appears well in advance of the other. Often two new body cells have been cut off by the spermatogenous cell before a body cell has both its blepharoplasts. The second blepharoplast arises in the same way but on the opposite side of the cell. Always they appear in the cytoplasm and at some distance from the nucleus. Although the stream lines are distinct in themselves, the cytoplasm in the neighborhood suggests a wavelike zonation, surrounding a narrow hyaline area in immediate contact with the blepharoplast (figs. 17, 23).

After the fusion of the granules, the blepharoplast remains a uniformly staining body for some time. Later the central area does not take the stain so deeply, but the margin remains dense. The stream lines have elongated considerably, and the zonation of the cytoplasm is still manifest. The blepharoplast has gradually approached the nucleus. At first the latter appears to beak slightly toward it (fig. 21); later the nuclear membrane becomes concave and the blepharoplast appears to lie in this hollow (fig. 23). Just before the division of the body cell the conditions are reversed. The nucleus is still hour-glass shaped, but the blepharoplasts occupy the farthest poles of the cell. The body cell is now quite spherical, but the blepharoplasts are flattened plates and are highly vacuolate (fig. 24). They occupy the opposite poles, and from them strong stream lines radiate. The vacuolated plate then seems to disorganize. Pieces are broken off which scatter in the cytoplasm (fig. 25). Each of these pieces seems to elongate and to develop protrusions, which at first are large and irregular, and then become ciliate (fig. 26). CALDWELL describes the blepharoplast as first giving rise to a distinct band which later may break up into fragments. This is not in accordance with my findings. Unfortunately the material at this stage was limited, and I was unable to decide whether the breaking up of the blepharoplast was a sign of the disorganization of the body cell, or the beginning of the formation of the ciliated band of the sperms.

The development varies considerably from the description of such stages in *Dioon* (3), *Stangeria* (5), *Ceratozamia* (4), and *Bowenia* (7).

Material for a more complete study of the transition from blepharoplast to ciliated band will be available when the cones, artificially pollinated by the writer in September 1927, are received in this laboratory.

**SPERM MOTHER CELL AND SPERM.**—CALDWELL has described briefly the mature body cell and the formation of the sperm. As only isolated stages of this later development are available, the present paper cannot add anything to this description.

### Discussion

The generative cell throughout the spermatophytes appears to be synonymous with the primary spermatogenous cell of the pteridophytes. Its product, the stalk cell, while sterile in all genera, is fertile in *Microcycas*; and it is probable that the four-sperm condition noted in *Ceratozamia* (4) may have resulted from the division of this cell. Further evidence of this potential fertility is produced by the situation in *Phyllocladus* (12) and *Dacrydium* (11). Here the generative cell divides into two collateral cells. In general one develops more rapidly than the other, resulting in a fertile body cell and a sterile stalk cell; but it has been recorded that both cells may develop into fertile body cells.

However, that all cases cited in the gymnosperms, where more than two male cells occur, are due to a revival of this latent activity of the stalk cell is very debatable. The stalk cell, and perhaps the body cell also, began its history as an active spermatogenous cell. An evolutionary tendency to a reduction in the number of archegonia in the female gametophyte, and of sperms or male cells in the male gametophyte, produced a gradual decrease in the activity of the spermatogenous cell, and it ultimately became sterile. This latter condition has been reached by all the living gymnosperms except *Microcycas*. In the Cupressineae, however, there is a reversion toward an increased number of archegonia inclosed in a common jacket, and a correlated increase in the number of male cells is therefore not unexpected. This situation has actually arisen in *Cupressus goveniana* (6) and *Juniperus communis* (8). By this time, however, the stalk cell is not only sterile but degenerate, as in many Cu-

pressineae no definite cell is organized. The increased activity is assumed by the body cell, therefore, and the large output of male cells is the result of the division of this cell. These cases are exceptional, however, and the evidence at present is so scanty that this explanation must be accepted as mere speculation.

One-half of this first generation of the primary spermatogenous or generative cell having become definitely sterile, it is not remarkable to find in the angiosperms a complete elimination of a generation of spermatogenous tissue, and the generative cell dividing directly to form male cells.

The linear arrangement of the body cells is concomitant with the configuration of the tube, and the latter is a condition resulting from the seed habit. The evidence of a transition from a spherical mass of spermatogenous tissue, such as we find in the antheridia of bryophytes and pteridophytes, to a linear arrangement of cells, as in *Microcycas calocoma* pollen tubes, could only be traced in ancient seed plants. Seeds of the Carboniferous era, where the male gametophyte has been at all preserved, suggest a spherical body with a comparatively large development of spermatogenous tissue. Just why the tube arose is a subject for speculation at present, but it carried in its wake a gradual elimination of spermatogenous cell divisions, except in one plane, and this gave rise to the uniseriate arrangement characteristic of the gymnosperms today.

The rise of the pollen tube is perhaps correlated with an increased separation in time of pollination and fertilization. In early forms the male gametophyte was probably shed from the microsporangium at complete maturity. The seed with its well developed pollen chamber received the microspore with its mature sperms, and the latter came to lie almost directly on the necks of the archegonia. An earlier shedding of the pollen would necessitate the further development of the male gametophyte taking place in the seed. To obtain nourishment the microspore developed a haustorium. Correlated with this early shedding of the microspore is an insufficiently developed pollen chamber, so that a tissue intervenes between pollen grain and archegonia. If the grain continues to be shed earlier and earlier in the life history of both gametophytes, a condition would arise comparable with that of the living gymnosperms.

### Summary

1. Pollination appears to take place during the last week in August and the first two weeks in September.

2. The pollen is shed in the three-celled condition, one prothallial cell, a generative cell, and a tube cell.

3. The generative cell divides, giving rise to a "stalk" cell and a body cell. The stalk cell is a fertile spermatogenous cell undergoing eight to ten successive divisions, each time cutting off a body cell. Centrosomes were at no time present in the spermatogenous cell.

4. The body cells divide once only, producing two-sperm mother cells. One exceptional case is described where a daughter cell, which normally would have become a body cell, remains spermatogenous.

5. The pollen tube moves through the nucellar tissue by solution of the cell walls and absorption of the cell contents.

6. There is only one prothallial cell.

7. The prothallial cell remains small until cessation of the activity of the spermatogenous cell. It then grows rapidly into and through the stalk or spermatogenous cell.

8. The spermatogenous cell remains turgid during the passage of the prothallial cell.

9. The blepharoplast appears in the young body cells as an aggregate of small granules.

10. After the enlargement and vacuolation of the blepharoplast, pieces are broken off which scatter in the cytoplasm of the sperm mother cell. This may be a process of disintegration.

11. Owing to insufficient material, the formation of the ciliated band could not be determined. A further study of this phase will be reported in a later paper.

12. The number of sperms ranges from sixteen to twenty.

In conclusion, I wish to acknowledge my indebtedness to Professor C. J. CHAMBERLAIN for suggesting the problem, and for his continual encouragement throughout the work, and to Professor W. J. G. LAND for helpful discussion and suggestions. I should also like to thank Dr. R. M. SAN PEDRO of Consolacion del Sur and friends in Cuba for their helpfulness in the field and their kindness in forwarding material.

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## EXPLANATION OF PLATES XIX-XXI

## PLATE XIX

FIG. 1.—Longitudinal section through integument, *int*; nucellus, *nu*; and pollen chamber, *pc*, showing reconstructed pollen tube (*t*), with its tube nucleus (*tn*); September;  $\times 100$ .

FIG. 2.—Longitudinal section of pollen grain as found in pollen chamber, September 15;  $\times 860$ .

FIG. 3.—Longitudinal section of pollen tube, tip of which has grown into nucellus, showing prothallial cell (*p*), generative cell (*g*), tube nucleus (*tn*); September 15;  $\times 860$ .

FIG. 4.—Longitudinal section of pollen tube; generative cell divided into "stalk" cell and first body cell; prothallial cell (*p*), spermatogenous cell (*s*), and body cell (*b*); September 21;  $\times 860$ .

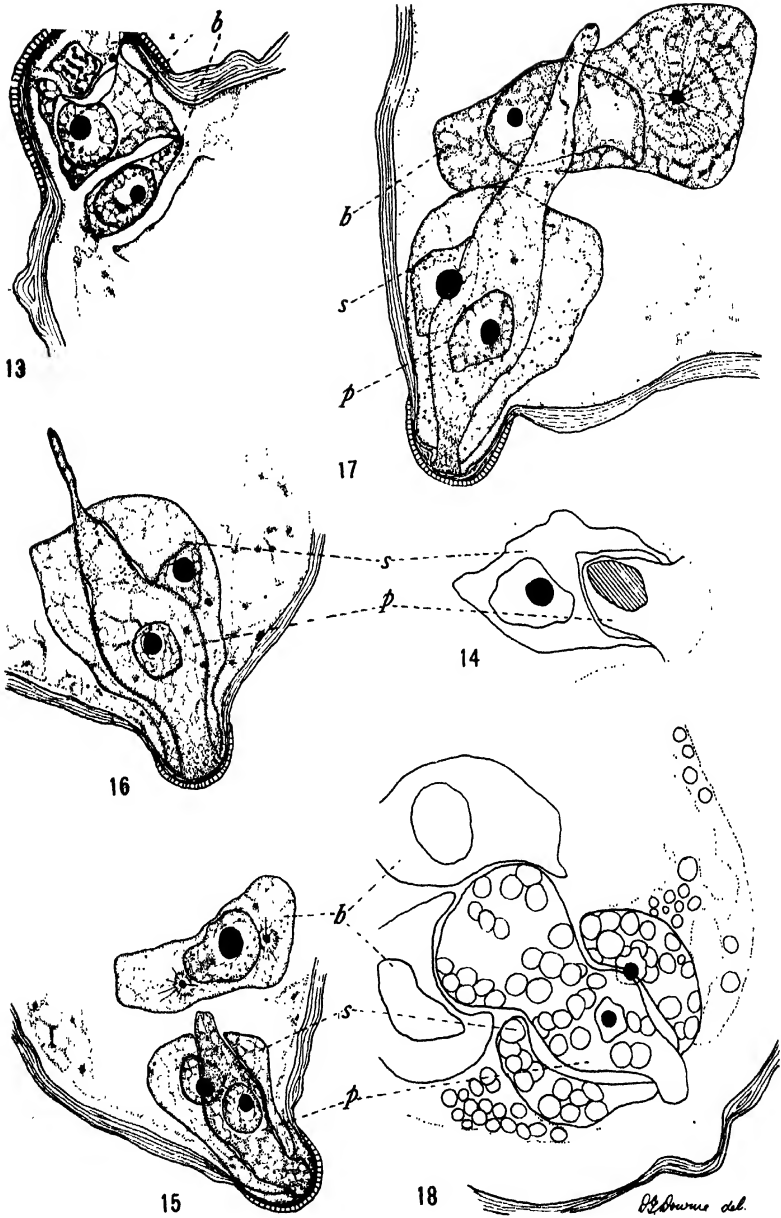
FIG. 5.—Section of pollen tube showing prothallial cell, spermatogenous cell, and body cells; division of spermatogenous cell just completed; September;  $\times 1580$ .

FIG. 6.—Section of grain end of tube showing prothallial cell, spermatogenous cell, and linear arrangement of body cells; September;  $\times 1260$ .

FIG. 7.—Longitudinal section of pollen tube showing prothallial cell (*p*), spermatogenous cell (*s*), and body cells (*b*); nucleus of spermatogenous cell in prophase; September;  $\times 1260$ .

FIG. 8.—Section of pollen tube showing prothallial cell (*p*), spermatogenous cell (*s*), and body cells (*b*); nucleus of spermatogenous cell in anaphase; September;  $\times 1260$ .





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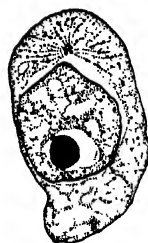




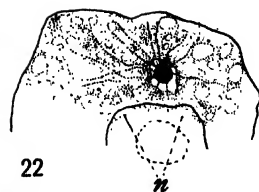
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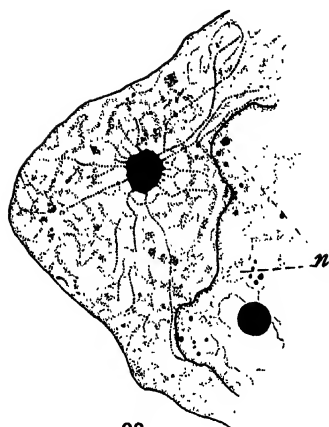


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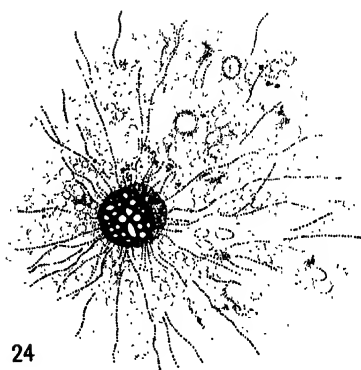


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## NUTATION IN *PINUS SYLVESTRIS*<sup>1</sup>

FRANK C. GATES

(WITH FIVE FIGURES)

During 1923 at Manhattan, Kansas, some curious differences of positions were noted on different days among the elongating shoots of a *Pinus sylvestris* which was being observed daily. This led to a series of observations and measurements during the four subsequent springs. The following paper is based on these observations.

The differences in position of the elongating shoots from day to day were very striking. Continuous observation for a few days showed too great a factorial regularity to be chance nutation. The factorial possibilities quickly reduced the causal effect to two principal ones; namely, wind and sun, both of which acted through differentiation of the growth by influencing the turgidity, as is possible by differentiation of the water balance. The bends or curves occurred only during a time of growth and ceased as soon as the longitudinal axis completed its elongation. The same effect was often seen on *Pinus banksiana* and a few times on *P. strobus* (fig. 1) in northern Michigan, but no trees of these species were available for daily observation. Two other pines, *P. laricio* and *P. ponderosa*, which were convenient for continued daily observation, showed no trace of this bend. In both of these cases the elongating stem was very stout, greatly contrasting with the slender, more rapidly elongating stems of *P. sylvestris*.

A priori, one might expect to find these tender shoots being blown with the wind, and consequently it was somewhat surprising not to find this more than once during the whole series of observations. This time was when a very strong wind, over 45 miles an hour, had full access to the tree (fig. 2). Aside from this the shoots were curved into the wind (fig. 3). The apparent mechanism was simply that of increased water loss on the side toward the wind, resulting in a forcing over into the wind because of the greater water pressure and

<sup>1</sup> Contribution no. 279, Department of Botany and Plant Pathology, Kansas State Agricultural College.

growth on the opposite side. Apparently the effect of sun was similar; the differentiation of the temperature brought about differentiation of the transpiration, causing differentiation of the water pres-

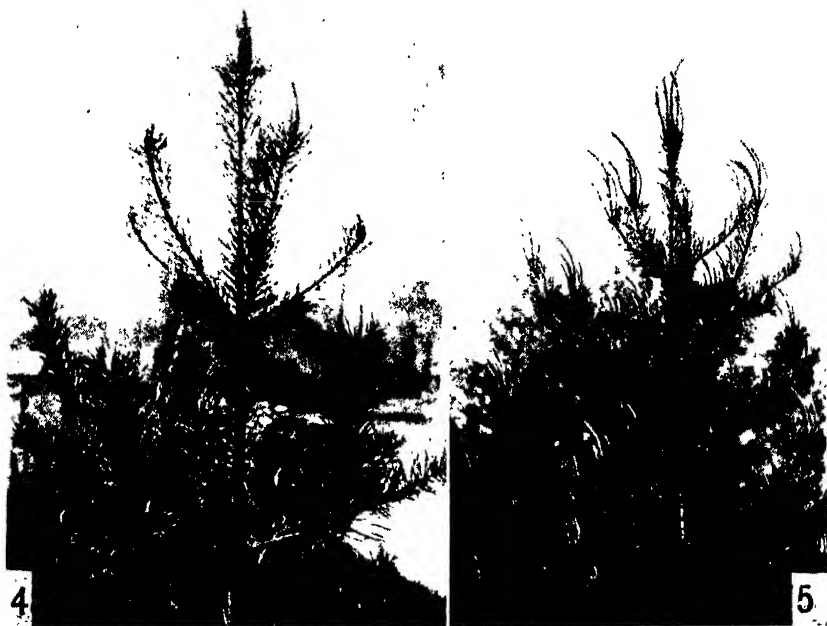


FIGS. 1-3.—Fig. 1, *Pinus strobus* at Douglas Lake, Michigan, showing shoots curved into the setting sun (June 26, 1927); fig. 2, *P. sylvestris* with topmost leader flying with southeast gale; all other shoots had made the change from northwest (fig. 3) into southeast with the change of wind; a little later in the day the topmost leader likewise curved into the southeast (April 20, 1925); fig. 3, *P. sylvestris* earlier on the same day as fig. 2; all shoots bent into strong northwest wind.

sure, bringing about growth curves into the direction of the sun. The actual result depended upon which was stronger; usually a wind strong enough to rustle the leaves of ordinary trees was suf-

ficiently strong to cause a curve into the wind, irrespective of the sun; with the air hardly stirring, curves into the sun were most conspicuous somewhat early in the day and again late toward night, suggesting positive heliotropism.

While there was a certain amount of variation, usually all leaders of the main stem and branches acted alike, and usually the whorl of



FIGS. 4, 5.—Fig. 4, *Pinus sylvestris* in virtually no wind, showing bending of leaders into sun shortly after sunrise (April 12, 1925); fig. 5, *P. sylvestris* after a rainy night accompanied by a sharp northwest wind; picture taken shortly after wind died down and sun came out of clouds in east; the more prompt response of topmost leader is shown (May 4, 1926).

branches at the base of the leader responded similarly. Variations were most likely to occur when the factors were less strong, and particularly when parts of the plant aided in preventing the operation of a factor, as, for instance, the north side shaded from the sun. In general the leaders were more active (figs. 4, 5). One would expect this from the fact that the leaders elongate more rapidly and to a greater degree than do the whorls of branches. During cool spells-

the amount of curving was checked and became more erratic, but also the amount of growth was greatly checked. Normally, rainy weather tended to increase the quantity of the response. In very light wind the extra water at first on one side would occasionally bring about responses away from the direction of the wind or rain, followed by irregular bends during the duration of the rain.

A few of the daily observations may be mentioned as samples.

#### 1924

April 21, 7:00 A.M.,  $10^{\circ}$  east into the sun, no wind; 3:30 P.M.,  $5^{\circ}$  northwest into the wind and sun, the sun seemingly of stronger effect as the bend did not occur until afternoon.

April 22, 7:00 A.M.,  $22^{\circ}$  northwest into wind opposite from sun.

April 24, 8:00 P.M.,  $30^{\circ}$  east into wind away from the setting sun.

April 25, 7:00 A.M., upright, a cloudy windless morning.

April 28, 10:00 A.M.,  $10^{\circ}$  east into sun, the plant really protected from a slight southwest wind; 8:00 P.M., followed sun across the sky and at sunset was  $45^{\circ}$  west; after sundown it straightened up to  $7^{\circ}$  west, no wind to speak of and that little did not strike the tree.

April 29, 7:00 A.M., following a light misty rain most of the late part of the night with a light easterly wind, leaders were bent to the west  $10-20^{\circ}$ , the curve being particularly conspicuous in growth region; 9:00 P.M., nearly erect at 6:00 P.M., but in the evening a  $20^{\circ}$  bend eastward into a gentle cool wind.

May 2, 6:00 A.M., mostly with a sharp  $30^{\circ}$  curve into the east to the sun, no wind; 8:00 P.M., after a light shower in the afternoon bent up to  $40^{\circ}$  into the sun at 5:40, but by 8:00 had straightened up to  $10^{\circ}$  northwest.

May 5, erect all day in strong southwest wind which did not hit the tree very sharply; toward night the wind died down and the leaders bent slightly to the setting sun; elongation now virtually completed, consequently bends less prominent.

#### 1925

April 7, elongation reached 61 mm. at this date, but no bends had been as yet noticed.

April 8, 7:00 P.M., slight bends for the first time noticed toward the west into the wind and sun.

April 12, 8:00 A.M., elongation now beginning to be rapid;  $10^{\circ}$  east before sunrise into a very light wind, changing to a more prominent bend into the sun (fig. 4) and following the sun across the sky to a  $10^{\circ}$  west bend at 6:00 P.M., virtually no wind all day.

April 15, 7:00 A.M., erect, a cloudy day.

April 16, 7:00 A.M., east into wind and sun  $20^{\circ}$ ; 7:00 P.M., west into sun  $10^{\circ}$  in spite of a light east wind.

April 17, 8:00 P.M., bends up to  $25^{\circ}$  east into a sharp breeze.

April 18, 6:00 P.M., after breeze during the day had died, the leaders erected and went over to as much as  $30^{\circ}$  northwest into the sun, although the light breeze remained southeast.

April 19, 8:00 A.M., a strong north wind during the night, and at daybreak leaders bent northwest into it in spite of the sun.

April 20, 1:30 P.M., a very strong northwest wind had had all the new growths bending into it as the sun came up (fig. 3), but when the wind changed to southeast and increased all morning to gale intensity, the growths were blown with it for about three hours, but at 1:30 only the uppermost leaders were with it (fig. 2), all others had curved  $10$ – $20^{\circ}$  into this southeast wind; sun obscured much of the time; by 5:30 the uppermost leaders had finally curved into this southeast wind.

April 25, 7:00 A.M., upright in a fog.

April 26, 7:00 A.M., slightly northwest into a cool northwest wind; needles now developing rapidly and elongation of stem had ceased and no further bends were noticed.

### Summary

1. A long series of observations on the elongating shoots of *Pinus sylvestris* showed that during the elongating period the new shoots are subject to nutations, which are caused by differentiation of the water pressure on the different sides, resulting in growth curves in the direction where the turgidity was least. The two agents that thus operated locally to decrease turgidity were direct sun and wind. In the absence of either, the shoots were erect.



2. The same effects take place regularly upon the slender-shooted *Pinus banksiana* and *P. strobus*, but were not present on the stout-shooted *P. laricio* and *P. ponderosa*. In general the wind seemed to have a greater effect than the sun, as long as it was strong enough to rustle leaves of ordinary trees. The effects were greatest when wind and sun operated together, but changes from one position to another following changes of the factors could be quite rapid.

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## PHYSICAL AND CHEMICAL CHARACTERISTICS OF EXPRESSED CITRUS LEAF SAP AND THEIR SIGNIFICANCE<sup>1</sup>

A. R. C. HAAS AND F. F. HALMA

It has been shown by HAAS (1) that the ash and calcium of the water-soluble fraction of mature normal Washington Navel and Valencia orange leaves are considerably greater than those of Eureka lemon leaves. In view of these differences, it would be logical to assume that differences would be found to exist in the freezing point lowerings of the expressed leaf sap, provided that carbohydrates are left out of consideration. It is the primary purpose of this paper to show the differences in the freezing point lowering of lemon and orange leaf sap. In addition to these fundamental physical differences between the leaf sap of lemon and that of orange, there are also chemical differences of considerable interest.

KELLEY and CUMMINS (4) have shown that the composition of the inorganic constituents of the ash of mature leaves is fairly constant in comparison with that of immature leaves, hence our studies were confined to normal leaves of one or more years of age, collected from 10-year-old trees growing on the grounds of the Citrus Experiment Station, Riverside, California. For each sampling one orange and one lemon tree growing in close proximity were selected.

It is generally recognized that the time of taking leaf samples for freezing point determinations is of utmost importance. This applies equally well to bark of shoots, as shown by HALMA (2). For this reason the leaves were collected on the shady side of the trees at approximately the same time, never later than 9:30 A.M. This procedure minimized the effects of sunlight on the concentration of the leaf sap, a point which will be discussed in a later paper.

The leaves were wiped free from dust, frozen for approximately 20 hours, and then ground in a meat chopper. The sap was expressed with a hand press. Realizing that the amount of pressure applied in

<sup>1</sup> Paper no. 177, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

obtaining the sap is an important factor, great care was taken to apply as uniform a pressure as possible to all samples. The errors which might have resulted from this source are negligible when considered in comparison with the large differences obtained. The freezing point of the sap was determined with the Beckmann apparatus, and corrected for under-cooling by the formula given by HARRIS and GORTNER (3). Determinations were made as rapidly as possible, and any remaining portion of the sap was used for the determina-

TABLE I  
FREEZING-POINT LOWERING ( $\Delta$ ) OF EXPRESSED LEAF SAP

DATE 1927	EUREKA LEMON	NAVEL ORANGE	VALENCIA ORANGE
	$\Delta$	$\Delta$	$\Delta$
August 16.....	1.331	1.446	1.402
August 19.....	1.208	1.471	1.496
August 25.....	1.283	1.469	1.437
August 30.....	1.239	1.531	1.437
September 13.....	1.387	.....	1.631
September 16.....	1.359	.....	1.801
September 20.....	1.317	.....	1.727
September 23.....	1.327	.....	1.694
September 26*.....	1.160	.....	1.566
October 13.....	1.334	.....	1.498
November 3.....	1.187	.....	1.397

\* Mature leaves of current season's growth from same trees as samples of September 20.

tion of ash, calcium, and magnesium by methods previously used by HAAS (1).

Table I shows that there are inherent differences in the physical characteristics in the sap of lemon and orange leaves. The lemon leaf sap was always more dilute than either Valencia or Navel orange, the smallest difference being  $0.071^{\circ}$  and the largest  $0.442^{\circ}$ . It will also be noted that there are no large or consistent differences between the values obtained for the Navel and Valencia saps, hence the investigation was largely confined to Valencia leaf sap. The absolute values obtained for the freezing point lowerings are by no means constant, but the important point to consider is that the values obtained for the two species always vary in the same direction and the difference is usually rather large. The results are especially

significant when it is considered that each determination represents samples from individual trees, and not a composite sample from many trees. It may be mentioned in passing that one of the factors which influences sap concentration is soil moisture; for example, after irrigation the sap tends to become more dilute. It is of interest in this connection to note that McCool and MILLAR (5, 6) found that the concentration of the soil solution is indicated by the freezing point lowerings of the root tissue, but found the tops to be far less sensitive to changes in concentration of soil solution. Their work

TABLE II

INORGANIC CONSTITUENTS OF 30 CC. OF LEAF SAP, EXPRESSED IN GRAMS

DATE 1927	EUREKA LEMON			NAVEL ORANGE			VALENCIA ORANGE		
	Ash	Calcium	Magnesium	Ash	Calcium	Magnesium	Ash	Calcium	Magnesium
August 16. ....	1.4923	0.4502	0.1027	1.6394	0.5070	0.0622	1.8774	0.6243	0.0669
August 19. ....	1.3753	0.4155	0.0888	1.7065	0.5385	0.0635	2.0688	0.7068	0.0704
August 23. ....	1.5753	0.4661	0.1163	1.8929	0.5872	0.0767	1.9957	0.6578	0.0720
August 25. ....	1.5115	.....	.....	.....	.....	.....	2.1926	.....	.....
August 30. ....	1.4373	0.4414	0.0956	1.8459	0.6056	0.0652	2.0630	0.7138	0.0716

was confined to annuals, however, and hence may not be applicable to evergreen trees.

The amounts of ash, calcium, and magnesium found in the leaf saps used in table I are given in table II. It is significant that the ash and calcium content of the sap of the lemon leaves are always lower than those of the orange leaves, a fact which confirms the results presented in table I. It may be pointed out in this connection that the ash and calcium content (table II) of Valencia leaf sap is consistently greater than that of the Navel orange. In this respect the data secured from finely ground dried leaf material differ from those obtained from sap extracts. An additional point of interest is the fact that the magnesium content of lemon leaf sap is higher than that of sap of orange leaves; in other words, an inverse relationship exists as compared with the calcium content. A possible significance of this difference in sap-soluble magnesium will be considered in another paper.

WEBBER and others (8) have recorded the well known fact that

lemon trees are more susceptible to injury by freezing temperatures than orange trees. The data presented in tables I and II suggest an explanation for this phenomenon. The sap of lemon leaves, being more dilute, would naturally permit the leaves to freeze at a temperature at which the orange leaves would not be injured, provided the trees are growing under similar environmental conditions. Moreover, the sap concentration of leaves taken from the shady side more nearly represents the sap concentration of the leaves at night, the time when low temperatures generally occur. As pointed out by SWINGLE and ROBINSON (7), the kumquat (*Fortunella*) is one of the hardiest of evergreen citrus fruit trees. Limited data actually show that its leaf sap is more concentrated than that of the Valencia. Discussion of the effect of environmental conditions upon the freezing point lowering of leaf sap in citrus must await future investigation.

### Summary

Fundamental differences exist in the freezing point lowering and in the ash, calcium, and magnesium content of leaf sap of Eureka lemon as compared with Valencia and Washington Navel orange. The sap of normal mature lemon leaves is less active osmotically and contains less ash and calcium, but more magnesium, than the sap of orange leaves. The fact that the sap of the lemon leaf is more dilute than that of the orange leaf furnishes a partial explanation of the differences in degree of sensitiveness to low temperatures between lemon and orange trees.

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## BRIEFER ARTICLES

### NOTE ON THE DECOMPOSITION OF SODIUM CYANIDE

During some recent investigations on the effect of various herbicides on plants, the writer performed some experiments with sodium cyanide to determine the rate at which it decomposed under various conditions. The results may be of interest, in view of the fact that the injurious after-effect of sorghum has been attributed to the formation of hydrocyanic acid by the sorghum residue. Sodium cyanide and hydrocyanic acid ought to decompose at approximately equal rates. When sodium cyanide is dissolved in water it hydrolyzes, forming hydrocyanic acid and sodium hydroxide. Such a solution has a very alkaline reaction. The reaction of a N/10 solution of sodium cyanide was calculated to pH 11.75.

Hydrocyanic acid and cyanides are described in textbooks of chemistry as unstable. By boiling an aqueous solution of potassium cyanide ammonium formate is formed. CLARK<sup>1</sup> found that a 24 per cent solution of potassium cyanide kept in the dark lost 75 per cent of its toxic value in ten days. BRENCHEY,<sup>2</sup> growing barley seedlings in solutions containing 2.5 parts per million of hydrocyanic acid, found the plants to overcome the poisonous action of the solution after about 4 weeks. This may be interpreted as a disappearance of the poison by that time. HAWKINS<sup>3</sup> found solutions of potassium cyanide toxic in concentrations of 1.0 ppm. At a concentration of 0.5 ppm, some plants were stimulated, however, which may be a result of decomposition of the salt. PETIT<sup>4</sup> found that small applications of various cyanogen compounds increased the yields of plants in pot cultures.

When a solution, corresponding to 100 ppm of sodium cyanide, was added to soil in pots in which barley, oats, or Canada thistles were growing, the plants were killed over night or in a few days. If the application was made some days before the seeds were planted, however, the seedlings grew better in the treated soil than in checks, indicating not only a rapid decomposition of the cyanide but that its nitrogen content had been

<sup>1</sup> CLARK, J. F., On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi. *BOT. GAZ.* 28:289-327; 378-404. 1899.

<sup>2</sup> BRENCHEY, W. E., Organic plant poisons. I. Hydrocyanic acid. *Ann. Botany* 31:447-456. 1917.

<sup>3</sup> HAWKINS, R. S., Hydrocyanic acid as a toxic agent for plant growth. *Jour. Amer. Soc. Agron.* 17:169-171. 1925.

<sup>4</sup> PETIT, A., Influence sur la végétation de quelques composés cyanogènes. *Jour. Agric. Pract. N.S.* 45:490-491. 1926.

made available for the plants. This observation prompted further investigation.

Several experiments were conducted in order to test certain factors that influence decomposition of NaCN. One of these was to test the influence of temperature on the decomposition of this salt. N/10 solutions were kept in darkness at 10°, 20°, and 30° C. for 40 days. Samples were withdrawn at regular intervals, and analyzed by titration with silver nitrate according to the Official Methods.<sup>5</sup> No decomposition of the salt was found in any of the samples. At the same time and under similar conditions, 200 cc. N/10 solutions was added to 100 gm. portions of soil in 300 cc. Erlenmeyer flasks. Aliquot samples were analyzed, at first daily and later at prolonged intervals. It was found that the concentration of the solutions, irrespective of temperature, decreased to about N/14 in 5 days. Later no changes took place up to 40 days, the time of the experiment. This result suggests that the cyanide was absorbed or adsorbed by the soil to a certain extent, but that no decomposition took place in a solution in contact with soil.

In order to test the decomposition under more natural conditions, solutions of sodium cyanide were added to 100 gm. portions of soil in amounts of 200 ppm of dry soil. Enough water was added to bring the soil to optimum moisture content. The samples were then kept at 10°, 20°, and 30° C. Daily one sample for each temperature was analyzed for cyanides. Two hundred cc. of water was added and the soil stirred. By suction through Buchner funnels fitted with three filter papers, clear solutions were obtained, which were titrated with silver nitrate. Soil samples kept at 10° C. gave a weak test for cyanide after 24 hours. Later analyses, as well as all analyses of samples kept at higher temperatures, gave negative results. The decomposition of the cyanide seemed to be very rapid. Finally sodium cyanide was added to soil in amounts from 50 to 200 ppm and barley sown immediately. It germinated in three days and made better growth in the treated than in the untreated soil. Thus the decomposition of sodium cyanide in soils is very rapid under "natural" conditions.

Since sodium cyanide decomposes so rapidly in soil, but not at all in a N/10 solution having a reaction of pH 11.75, it indicates that the decomposition is brought about by microorganisms.

It is not likely that a compound decomposing as rapidly as hydrocyanic acid is the cause of the injurious after-effect of sorghum.—ALFRED ASLANDER, *Cornell University, Ithaca N.Y.*

*[Accepted for publication December 2, 1927]*

<sup>5</sup> DOOLITTLE, R. E., ET AL., Official and tentative methods of analysis, etc. 2d ed. . pp. 535. Washington.



# CURRENT LITERATURE

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## BOOK REVIEWS

### Symbiontism and origin of species

A new theory of the evolutionary origin of species is proposed by WALLIN,<sup>1</sup> who has for some years believed that mitochondria should be interpreted as symbiotic bacteria. The introductory chapter discusses the three cardinal features of evolution: the origin of species, the survival of species, and the direction of evolution. DARWIN's principle accounts for the preservation of suitable organisms, but does not account for the production of evolutionary variations. The author of this book thinks that symbiontism does account for the evolutionary development of new species.

In the second chapter he discusses the history of mitochondrial research, from ALTMANN's original work in 1890 to the present time, and then proceeds to a discussion of the bacterial nature of the mitochondrial bodies. This theory rests upon staining reactions in part, upon reactions to physical and chemical agents, upon thermal responses, and upon cultural studies made with tissues. The main cultural studies were made with liver tissue from new born or fetal rabbits, explanted to a rabbit liver meat infusion medium. Pieces of fat, kidney, and suprarenal tissue from rabbits, guinea pigs, dogs, and cats were used also, with the production of growths which lead WALLIN to state: "These facts, apparently, admit of no other interpretation than that mitochondria are living organisms, symbiotically combined with the cells of plants and animals." That is a large conclusion to draw from about ten pages of discussion, which contains only a small amount of experimental evidence. The foundation seems to the reviewer too thin to bear the weight of superstructure that rests upon it.

Chapter IV takes up the behavior of mitochondria, pleomorphism, transformation into glandular secretions, experimental modifications, relation to disease, to cell division, and neoplastic growths. The author takes these activities as those of symbiotic bacteria, and proceeds to define symbiontism in the following chapter.

This term he defines as an "absolute intracellular symbiosis" resulting from positive prototaxis. In the author's words: "Mitochondria represent one of the symbionts in the expression of a positive prototaxis resulting in an absolute symbiosis. The universal presence of mitochondria in the cells of all plants and animals constitutes evidence that the development of such relationships is not

<sup>1</sup> WALLIN, I. E., *Symbiontism and the origin of species*. 8vo. pp. xii+171. Baltimore: Williams and Wilkins. 1927.

a digression from normal biological behavior, but rather the result of a fundamental principle. The universality of positive prototaxis resulting in absolute symbiosis, or symbiointicism, forces us to recognize this as of fundamental importance. Just as reproduction insures the *perpetuation of existing species*, the author believes that *symbiointicism insures the origin of new species*."

The sixth chapter deals with some cases of known microsymbiosis. Included among these are such forms as *Amoeba viridis*, sponges, *Hydra viridis* and other *Coelenterata*, turbellarian worms, and other forms inhabited by such algae as the *Zoochlorellae*, *Zooxanthellae*, etc. Cases of bacterial microsymbiosis include *Pelomyxa vivipara* among the Protozoa; such insects as *Blatta germanica*, *Periplaneta orientalis*, *Camponotus ligniperda*, *Formica fusca*; and certain bioluminescent animals, such as squids, whose luminiferous organs contain luminescent bacteria.

Chapter VII analyzes the symbiont relations, and then the author attempts to show that symbiointicism is responsible for the origin of species. The final paragraph of chapter VIII expresses WALLIN's convictions. "It appears to the author that symbiointicism offers a rational explanation for many of the variations in the morphology and physiology of plants and animals. When these variations are of sufficient magnitude and permanence, they constitute new species. The facts that mitochondria are universally present in the cells of all organisms higher than the bacteria, that mitochondria are bacterial in nature, and that microsymbiosis can determine morphologic and physiologic changes in organs and cells, can lead to no conclusion other than that *symbiointicism is a fundamental causative factor in the origin of species*."

The last two chapters consider the relation of symbiointicism to heredity and development, and to organic evolution in general. WALLIN tries to fit his theory to the existing theory of Mendelian behavior. Rejecting the idea that evolution is due to the loss or disintegration of genes, but must be accounted for by the acquisition of new genes, he proposes the idea that the acquisition of a new symbiont in the cells of an organism may be the means by which new genes are added to the germ cells. The actual formation of the gene is pictured as the transfer of the chromatin material of the bacterial symbiont to the germplasm of the host. That is, on entering the cell, the chromatin scattered through the bacterial body is supposed to leave it and pass to the chromatin material of the host cell nucleus. The bacterial body, with its chromatin gone, remains in the cytoplasm, and becomes by subsequent divisions the mitochondria of the host cell cytoplasm. In the nucleus of the host cell, the chromatin of bacterial origin is supposed to take its place in the chromosome as a new gene, and to enter upon the expression of new characters on its own account. Also, by modification of other genes already present, the new gene may have many complex effects in the realm of hereditary variations.

The final chapter leaves one unsatisfied, because there is lacking anything concrete in the application of symbiointicism to the actual evolution of life upon the earth. It merely gives the author an opportunity to emphasize the difficul-

ties of all theories to explain the origin and direction of the evolution of higher organisms.

The work presents a unique point of view, very insufficiently supported. Many able scientists have studied the mitochondria without catching this view of their significance. If WALLIN were to be found correct in his interpretation, the work would open up many excellent lines of research. It takes a man of courage to publish a theory which he knows will be by some rudely discarded, and made by others the center of hot controversy in which he may be worsted. The discussion is interesting, and even if it is unconvincing, it should gain for its author a respectful hearing. The very lack of convincingness may stimulate work on the part of those who do not agree with WALLIN's interpretation, to the end that proof of the correctness or incorrectness of the basic observations may be brought forward. It should be relatively easy to determine whether the evidence of chapter III is valid.—C. A. SHULL.

#### Biochemical laboratory methods

At the time of his death, the late Professor MORROW<sup>2</sup> of the University of Minnesota left an unfinished manuscript of a laboratory manual of biochemical methods, which has been brought to completion through the efforts of Mrs. MORROW, with the assistance of Dr. R. A. GORTNER and T. A. PASCOE of the Division of Agricultural Biochemistry. The book is designed particularly for students of the biological sciences, and it seems to the reviewer a satisfactory manual, particularly for the non-medical groups.

The experiments cover a wide range of procedures, and anyone who masters the technique outlined in this work will be well grounded in the fundamentals of biochemical methods. There are 233 experiments outlined, which are grouped into nine chapters. The first deals with the colloidal state, and offers 65 studies of dialysis, diffusion, osmosis, optical properties of colloidal matter, preparation of sols, emulsions and emulsoids, electrical properties, ultra-filtration, viscosity, surface tension, surface energy, adsorption, and related topics.

Chapter II presents several exercises on the physical chemical constants of plant saps, and the following chapter considers the measurement of hydrogen-ion concentration, and buffer phenomena. The fourth chapter deals with the proteins, and offers forty valuable experiments which lead up to the analysis of a protein for its principal nitrogen fractions. The following chapter gives thorough consideration to the carbohydrates. Thirty-six of the exercises deal with the soluble monosaccharides and disaccharides, and twenty with the polysaccharides.

The four remaining chapters are less extensive, and treat the glucosides, fats and allied substances, enzymes, and plant pigments. The enzyme experiments cover the proteases and amidases, carbohydrases, glucosidases, esterases, oxidases, and catalase.

<sup>2</sup> MORROW, C. A., *Biochemical laboratory methods*. 8vo. pp. xviii+350. New York: Wiley and Sons. 1927.

Literature citations accompany the experimental directions, and provide a satisfactory background of biochemical theory for the student's work. A book of this kind has long been needed; it should find wide use among those preparing for physiological and biochemical research.—C. A. SHULL.

#### Factors controlling germination and early survival in oaks

A study of the problems connected with germination of oaks and the early survival of seedlings of the various species of *Quercus* has been made by KORSTIAN,<sup>3</sup> of the Appalachian Forest Experiment Station. The study included conditions of successful storage, factors influencing the germination, such as biotic factors, moisture conditions, temperature, pericarp difficulties, character of food reserves, size of seeds, and certain soil conditions, like compactness of surface, and the influence of leaf litter on the retention of soil moisture and on the retention of viability of the seeds.

The loss of seeds through the ravages of insects, and the agency of rodents and other animals, is very large, 50–100 per cent in some cases. Retention of viability depends partly upon retention of water in the seeds. Red oak acorns are slightly drier than those of the white oak, the usual percentage of water being 50–60 per cent for the former, and 60–70 per cent for the latter species. If the acorns dry out there is a large decrease in viability by the time the moisture content reaches 20–30 per cent, and complete loss if the water content falls as low as 10–15 per cent. Proper storage must provide sufficient atmospheric or soil moisture to maintain the general high level of water content in the seeds, and at the same time provide enough oxygen for the respiration of the moist acorns.

The most favorable temperature in general is 50–65° F., low at night and higher in the day time. Below 40° germination does not occur, and at 80–95° night and day temperature, the temperature affects the germination unfavorably.

There is some delay of germination among the black oak group, a group whose seeds are more fatty than those of the white oak group. The presence of a rest period, and high-temperature hastening of germination, is believed to be associated with enzyme activity, particularly with reference to the conversion of fats to carbohydrates.

Forest fires usually destroy the viability of acorns lying under the leaf litter. The species or groups differ in their resistance, the red oak acorns being most resistant. In order of decreasing resistance to high temperature, the groups stand as follows: red oak, chestnut, black, scarlet, and white. As in many other cases, the larger acorns give more vigorous seedlings and better percentage of germination than the smaller seeds.

One of the edaphic factors of importance is the compactness of the surface soil. Studies of the soil with a penetrometer, coupled with studies of the ability

<sup>3</sup> KORSTIAN, C. F., Factors controlling germination and early survival in oaks. Yale Univ. School Forestry, Bull. 19. pp. 115. New Haven. 1927.

of the oak seedlings to penetrate the substratum, lead to the conclusion that the limit of soil penetration for oak radicles occurs with a surface penetrability of 0.01–0.023 mm. per gram of load applied.

Leaf litter is of the greatest importance in producing an environment suitable to germination and survival, because it stabilizes the moisture and temperature conditions, and prevents compacting of the surface soil beyond the limits of root power of penetration.

It is a pleasure to see forestry research turning toward the more fundamental physiological problems of tree life. There must be a much stronger movement in this direction if forestry is to become something else than a superficial branch of botanical science.—C. A. SHULL.

### Physical chemistry and biophysics

An attractive textbook on physical chemistry and biophysics for students of the biological and medical sciences has been prepared by STEEL.<sup>4</sup> It is well written, intensely interesting, and more practical than most books of the kind. It gives exactly the kind of information that students need for an understanding of the fundamental physical chemistry of cellular physiology.

The book contains thirteen chapters, the first of which is a brief introduction outlining the background of the modern development of physical chemistry in the service of biology. Chapter II considers the nature and structure of matter, atomic theory and recent advances in knowledge of atomic structure, radiations, radioactive substances, isotopes, atomic numbers and X-ray spectra, the laws of chemical reactions, etc. This is followed by a chapter on the general properties of matter, states of aggregation, gas laws, and deviations in behavior, liquids, viscosity and fluidity, crystalline state, and crystal systems.

The fourth chapter considers the energy transformations in living matter with reference to the laws of thermodynamics. The storage of potential energy in living matter, the energy changes of photosynthesis, and energy changes in animals are some of the topics discussed. Chapters V–VIII take up the general nature of solutions, water as a solvent, diffusion and osmotic pressure, and the nature and behavior of electrolytes in solution. The discussion of osmotic pressure is somewhat meager, but few exceptions can be taken to the statements made.

The later chapters take up the problems of chemical equilibrium and the law of mass action in physiological processes, measurement of hydrogen-ion concentration, colloidal state of matter, catalysis and the velocity of chemical reactions, and the dynamical physical chemistry of the cell. The chapter on the colloidal state is by far the longest in the book, and is a valuable treatment of the subject for the student.

The reviewer considers this book one of the best of its kind. It is worthy of

<sup>4</sup> STEEL, M., *Physical chemistry and biophysics*. 8vo. pp. x+372. New York: John Wiley and Sons. 1928.

a place on the young biologist's five-foot bookshelf. Even those who are not specializing in physiological fields would do well to know this book. The capacity to carry on worth-while investigations in any biological field will be enlarged by possession of the information presented in such a book.—C. A. SHULL.

### Flora of the Panama Canal Zone

STANDLEY<sup>5</sup> has published a very important contribution to our knowledge of the flora of the Panama Canal region. In 1921 the governor of the Canal Zone asked the assistance of the Department of Agriculture in the preparation of an illustrated flora of the region. STANDLEY was selected to prepare the flora, and the results of his investigations are now published. The purpose of the investigation was not only to contribute to our scientific knowledge of the region, but also to develop our knowledge of tropical agriculture. Some idea of the method of presentation may be obtained from the statement that "with the aid of the many English and Spanish common names, it should not be difficult for the casual visitor to identify most of the important plants of the Canal Zone."

The flowering plants of the region number about 2000 species. Besides the native plants, the keys include also the cultivated species. The work is based on the collections of the United States National Herbarium, which for many years has been receiving a wealth of material from collectors. STANDLEY himself visited the Canal Zone twice, studying the flora and making collections. He gave special attention to the common names in use, and also to the local uses of plants. The publication is not merely a taxonomic account of the flora, therefore, but includes also much material of more general interest.

An interesting account is given of the contrasts in physiography and flora of the Atlantic and Pacific slopes. About two-thirds of the Canal Zone lies on the Atlantic slope, and has a highly diversified flora, very little of the pioneer vegetation remaining. The Pacific slope is comparatively arid, and the vegetation is very different in type from that of the Atlantic slope. This difference in the two floras consists not only in the general appearance, but also in the genera and species represented. The most conspicuous element of the Atlantic slope flora is the great number and variety of palms. The author also includes a very interesting account of the history of botanical exploration in Panama. In short, the publication assembles in a single volume all of the available information in reference to the development of our knowledge of a very interesting region. It will also make it possible to appreciate and investigate more intelligently the closeness of the relationship of this flora to that of South America. The 66 remarkably fine plates picture some of the outstanding features of the flora.

Only about 50 pages are taken up with the general presentation of the

<sup>5</sup> STANDLEY, PAUL C., *Flora of the Panama Canal Zone*. Contrib. U.S. Nat. Herb. 27: pp. 416. pls. 66. 1928.

region, its history and its outstanding features. The bulk of the volume is made up of the taxonomic presentation of the flora, and presents the facts in simple and readable form. Detailed descriptions are not given, but there are keys for identification that will help the taxonomist. These keys include not only all the native or naturalized species, but most of the introduced plants grown for ornament or for economic purposes. In the case of the more important plants, those likely to prove of the greatest interest to the public, more extensive accounts are given which will assist in recognizing the species. It is not a rigidly taxonomic work in the usual sense, therefore, but a general introduction to the flora of an interesting region, which may be of service not only to botanists, but also to all who may be interested in the region.—J. M. C.

#### Flora of the Chicago region

In spite of the fact that for more than quarter of a century Chicago has been a center of botanical research, the region has been without any adequate check list until the present volume by PEPOON.<sup>6</sup> This is much more than a check list, as it contains many things besides an annotated list of more than 1900 of the vascular plants of the region. The 115 illustrations serve to enliven the text and assist in the recognition of many species. This recognition is further aided by 25 simple keys for groups and families. These include one for the trees in winter condition, illustrated with pen-and-ink sketches of 50 twigs and buds.

The recent geological history of the region is sketched, and on a topographic and ecologic basis six distinct floral districts are outlined. Each of these is mapped and its vegetation discussed in a separate chapter. This furnishes a historical background of the original plant associations that will be of permanent value. The good printing and binding, the excellent photographs, well produced, the careful organization, and the adequate index all add to the value of the book.—G. D. FULLER.

#### Investigations on chlorophyll

This classic work by WILLSTÄTTER and STOLL has been translated into English by SCHERTZ and MERZ,<sup>7</sup> both of the United States Department of Agriculture. The translation is fairly faithful, and many students will be pleased to use the work in this form. It is too well known to need description, but has not been well enough known to those who might want to make use of the methods WILLSTÄTTER developed. The price of the book is \$4.50, and can be obtained only from Dr. F. M. Schertz, 1305 Farragut St. N.W., Washington, D.C.—C. A. SHULL.

<sup>6</sup> PEPOON, H. S., *An annotated flora of the Chicago region*. Chicago Acad. Sci. Bull. Nat. Hist. Survey 8: pp. xxii+554. figs. 115. 7 maps. 1927. \$3.50.

<sup>7</sup> SCHERTZ, F. M., and MERZ, A. R., *Investigations on chlorophyll*. WILLSTÄTTER and STOLL. 8vo. pp. xii+385. Science Press Printing Co., Lancaster, Pa. 1928.

## NOTES FOR STUDENTS

**Taxonomic notes.**—KECK<sup>8</sup> has published a revision of *Orthocarpus*, which restricts the genus to the annuals, the longer-lived ones being incorporated in *Castilleja*. In addition to the ordinary enumeration of species, the author has constructed a suggestive phylogeny, illustrated by a chart. The genus as defined includes 25 species and 23 varieties, 4 of the latter being new.

BROTHERUS<sup>9</sup> has published an account of the known species of the mosses of Hawaii. The number of representatives of this group, both in species and genera, is remarkable. There are 84 genera recognized, including 139 species, 45 of which are described as new.

HENRARD,<sup>10</sup> in continuation of his revision of *Aristida*, which is a preliminary study to a monograph, has presented and discussed 168 species, 24 of which are described as new. There are many changes in nomenclature, and some of the species include numerous varieties. The species are presented in alphabetical order, the present paper extending from *A. hassleri* to *A. prodigiosa*. This will give some indication of the size of the genus.

MAIDEN<sup>11</sup> has published two additional parts of his revision of *Eucalyptus*. The first paper deals with *E. bloxsomei*, a new species, and presents in great detail its distribution and the factors that influence it, the problem of age and area, etc. In the second paper 10 species are discussed, followed by many details relating to the effective study of *Eucalyptus*, and its importance in numerous phases of industry and education.

PRAIN and BURKILL<sup>12</sup> have published an account of a collection of species of *Dioscorea* obtained from Siam. The species are said to belong to various floras that meet in Siam. The paper contains descriptions of 32 species, in 6 sections of the genus, 8 of which are described as new. There are also 6 new varieties.

SMALL<sup>13</sup> has described a new palm, *Sabal jamesiana*, discovered in the Everglades of Florida. He calls attention to the fact that Florida may well be called the "land of palms," since 13 of the 16 species occurring in the United States are found there.

<sup>8</sup> KECK, D. D., A revision of the genus *Orthocarpus*. Proc. Calif. Acad. Sci. 16:517-571. 1927.

<sup>9</sup> BROTHERUS, V. F., Hawaiian mosses. Bernice P. Bishop Museum, Bull. 40. pp. 37. pls. 8. 1927.

<sup>10</sup> HENRARD, J. T., A critical revision of the genus *Aristida*. Mededeelingen Van's Rijks Herb. Leiden. no. 54A. 221-464. 1927.

<sup>11</sup> MAIDEN, J. H., A critical revision of the genus *Eucalyptus*. Vol. VII. part 7. Publ. by Government of New South Wales, Sydney, Australia. 1926. Vol. VII. part 8. *ibid.* 1927.

<sup>12</sup> PRAIN, D., and BURKILL, I. H., The genus *Dioscorea* in Siam. Kew Bull. no. 6. 1927.

<sup>13</sup> SMALL, J. K., The James Palmetto, *Sabal jamesiana*. Jour. N.Y. Bot. Gard. 28:181-185. 1927.



BLAKE<sup>14</sup> has published a new revision of *Diplostegium*, the former one having been published in 1922. It was called for by the securing of new material and information. The genus is closely related to *Aster*, and the author regards it as "an Andean development of *Aster*." It is a South American genus, with the center of its abundance in Colombia. The new revision includes 43 species, 7 of which are described as new.

KOBUSKI<sup>15</sup> has described a new genus (*Apassalus*) of Acanthaceae. It includes three species separated from *Dyschoriste*. These species occur in Haiti and Santo Domingo, Cuba, and southeastern United States.

KOBUSKI<sup>16</sup> has published a monograph of the American species of *Dyschoriste*, based upon a study of the material contained in the principal American herbaria. Preceding the description of the species, he presents an account of the history, the general morphology, the geographical distribution, and the phylogeny of the genus. The recognized species are 40 in number, 12 of which are described as new. There is also a full record of all the specimens examined.

Miss MATHIAS,<sup>17</sup> in beginning the publication of her studies of the Umbelliferae, has presented a full account of the maritime genus *Glehnia*, disentangling it from its previous taxonomic treatment. Two species are recognized, which are closely related, but they occur along the coast on both sides of the Pacific. One species extends from Alaska to northern California, and the other from Siberia to southern China and through Japan.

In continuation of his studies of the Fabaceae, RYDBERG<sup>18</sup> has presented the genus *Atelophragma*. This genus was established in 1906, based on 6 species of *Astragalus*. In the present paper 22 species are recognized, distributed through 7 sections. Six species are described as new, and 8 are transferred from other genera. In another paper<sup>19</sup> he continues his presentation of the genus, describing 21 species, seven of which are new. The total number of species he has now published in the genus is 46.

RYDBERG<sup>20</sup> has also published some of the results of his investigation of *Psoraleae*, a tribe of Fabaceae. He characterizes 19 genera in the key, and gives an account of the first 8 of them in the present paper.

<sup>14</sup> BLAKE, S. F., Review of the genus *Diplostegium*. Amer. Jour. Bot. 15:43-64. 1928.

<sup>15</sup> KOBUSKI, C. E., A new genus of Acanthaceae. Ann. Mo. Bot. Gard. 15:1-8. 1928.

<sup>16</sup> ———, A monograph of the American species of the genus *Dyschoriste*. Ann. Mo. Bot. Gard. 15:9-90. 1928.

<sup>17</sup> MATHIAS, MILDRED E., Studies in the Umbelliferae I. Ann. Mo. Bot. Gard. 15:91-108. 1928.

<sup>18</sup> RYDBERG, P. A., Notes on Fabaceae. X. Bull. Torr. Bot. Club 55:119-132. 1928.

<sup>19</sup> ———, Notes on Fabaceae. XI. Bull. Torr. Bot. Club 55:155-174. 1928.

<sup>20</sup> ———, Genera of North American Fabaceae. III. Tribe *Psoraleae*. Amer. Jour. Bot. 15:195-203. 1928.

RUSBY<sup>21</sup> has published a new genus (*Mulfordia*) of Zingiberaceae from the marsh region of Bolivia. Only a single specimen was collected, which at first was thought to belong to *Costus*.

TRELEAVE<sup>22</sup> has published 4 new species of *Peperomia* from Bolivia. OSTERHOUT<sup>23</sup> has described 3 new species from Colorado, belonging to the following genera: *Quamoclidion*, *Astragalus*, and *Townsendia*.

MISS DREW<sup>24</sup> has published a revision of a group of small filamentous Rhodophyceae on the Pacific Coast. The discussion of the relationships shows that the species of *Chantransia* and *Acrochaetium* should be referred to *Rhodochorton*. Under that generic name she describes 34 species, among which there are 18 new species, 11 new combinations, and one new variety.

BLAKE<sup>25</sup> has described 12 new species of Compositae from Mexico and some of the northern countries of South America. They include 4 species of *Verbesina*, 2 of *Gynoxys*, and 1 each in 6 other genera.

SPRAGUE<sup>26</sup> has described a new genus (*Diacrodon*) of Rubiaceae from Brazil, from specimens that were assigned originally to the genus *Borreria*. It belongs to the tribe Spermacoceae, and seems to be a very distinct genus.

MUNZ<sup>27</sup> has published a revision of *Chylismia*, a subgenus of *Oenothera*, based on his study of the collections in many herbaria. Eight species are recognized, and 14 varieties, 3 of the latter being described as new. Some idea of the complexities of the group may be obtained from the fact that there are 6 new names for varieties, and 4 new combinations. The descriptions are very complete, and all the known collections are cited.

MUNZ<sup>28</sup> has published an elaborate revision of *Antirrhinum* and related genera, as represented in the "New World." The descriptions are very full, and all the localities from which material has been obtained are noted. Seven genera

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<sup>21</sup> RUSBY, H. H., *Mulfordia*, a new genus of the Zingiberaceae. Bull. Torr. Bot. Club 55:165, 166. 1928.

<sup>22</sup> TRELEAVE, WILLIAM, New species of *Peperomia* from Bolivia. Bull. Torr. Bot. Club 55:169-170. 1928.

<sup>23</sup> OSTERHOUT, G. E., New plants from Colorado. Bull. Torr. Bot. Club 55:75, 76. 1928.

<sup>24</sup> DREW, KATHLEEN M., A revision of the genera *Chantransia*, *Rhodochorton*, and *Acrochaetium*. Bot. Publ. Univ. Calif. 14:139-224. 1928.

<sup>25</sup> BLAKE, S. F., Twelve new American Asteraceae. Jour. Wash. Acad. Sci. 18:25-37. 1928.

<sup>26</sup> SPRAGUE, T. A., *Diacrodon*, a new genus of Rubiaceae from Brazil. Kew Bull. Miscell. Inf. no. 1. 32-34. 1928.

<sup>27</sup> MUNZ, P. A., A revision of the subgenus *Chylismia* of the genus *Oenothera*. Amer. Jour. Bot. 15:223-240. 1928.

<sup>28</sup> ———, The Antirrhinoideae-Antirrhineae of the New World. Proc. Calif. Acad. Sci. 15:323-397. 1926.

are included, one of which (*Epixiphium*) is new. The species are 45 in number, the large genera being *Linaria* (15 species) and *Antirrhinum* (14 species).—J. M. C.

**Hydrogen ion concentration of certain western American inland waters.**—SHELFORD<sup>29</sup> has reached the conclusion that hydrogen ion concentration is related to salt content of the water and to the amount of decomposition of organic matter. The bicarbonates are usually the measure of buffer value, but this is influenced somewhat by other alkaline salts. The decomposition of organic matter, respiration of organisms, etc., yield  $H_2CO_3$ . A water with much buffer will neutralize the  $CO_2$  from the decomposition and respiration of many organisms and still remain alkaline. A water with little buffer would become acid under the same conditions. Heavy rains of rainy periods in dry and moderately rainy regions and of regions of heavy rainfalls increase the hydrogen ions by diluting the buffer.

Where two streams meet, as the Columbia and Snake rivers of this study, differences in condition are due to age of waters, etc. With two waters of unequal age, four or more differences occur: in hydrogen ions, in temperature, and in quantity and quality of dissolved content. The effects of hydrogen ion concentration on the distribution of animal life in the water are modified by the amount of oxygen, quality of salts, etc., dissolved in the water.

There are two principal difficulties involved in hydrogen ion determination under the conditions of this investigation; one is the loss of  $CO_2$  due to contact with the air, and the other is the failure to get the sample from the place where the organisms in question live.—J. ISENBARGER.

**North American Flora.**—The first part of volume 23 contains the beginning of a presentation of the Mimosaceae by N. L. BRITTON and J. N. ROSE, including the Ingeae, the first of the six tribes. It contains 16 genera, all but the last one (*Lysiloma*) being presented. The following new genera are described: *Jubunba* (from *Acacia*) with 15 species, *Punjuba* (from *Pithecolobium*) with 1 species, *Cojoba* (from *Mimosa*) with 17 species, *Painteria* (from *Pithecolobium*) with 4 species, *Chloroleucon* (from *Pithecolobium*) with 9 species, and *Pseudalbizzia* (from *Acacia*) with 1 species. Much the largest genus is *Anneslia*, with 113 species, 46 of which are described as new. Distributed among the other genera are 28 new species.—J. M. C.

<sup>29</sup> SHELFORD, V. E., The hydrogen ion concentration of certain western American inland waters. *Ecology* 6: 279-287. 1925.

## GENERAL INDEX

Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold-face** type; synonyms in *italics*.

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